

UNIVERSITY OF BRISTOL



THE ANNUAL REPORT

OF THE

Agricultural and Horticultural
Research Station

(THE NATIONAL FRUIT AND CIDER INSTITUTE)

LONG ASHTON, BRISTOL
1949

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INTRODUCTION.

By T. WALLACE, C.B.E., M.C., D.Sc., F.R.I.C., Director.

1949 has witnessed further steady progress in the post-war programme of investigations. Close liaison with the National Agricultural Advisory Service has been maintained along the lines described in the previous report. Some advisory enquiries continue to be received at the Institute both on farm and garden problems and the usual procedure has been to pass on the former to the local N.A.A.S. headquarters and the latter to the County education centres. Enquiries from Bristol are referred to Mr. Cutler, the City Horticultural Officer. Since the submission of general advisory problems to the Institute often involves delay and extra expense for postage in passing on the enquiries to the appropriate centres, it is hoped that members will help by making the normal advisory procedures still better known to farmers and the general public. The Institute, of course, still functions as an advisory centre for problems relating to the manufacture of cider and fruit juices, and its help is also available to all advisory officers on problems on which the staff can provide specialist knowledge. It is hoped that in future the contacts of the Institute with members and the advisory services will be still closer than hitherto, since it is proposed to establish a Technical section to achieve this end. Agreement by the Treasury and the Agricultural Research Council has already been obtained to the appointment of an officer to organise this section but unfortunately the scheme cannot be put into operation at present owing to lack of accommodation.

The Cider Advisory Committee and its two sub-committees all held meetings during the year. The main committee, at its meeting on May 5th, adopted in its final form the scheme for the establishment of trial bush and standard orchards of the approved cider varieties. The scheme lays down the conditions to which recipients of trees will be asked to agree to ensure that the orchards will serve the purposes for which they are to be established. The detailed scheme was subsequently circulated to various organisations likely to be interested, including the N.A.A.S., subscribing County Councils and the National Farmers Union, and also to members particularly interested in cider orcharding, with a view to obtaining their co-operation in the work. Since circulating the particulars many offers of sites have been received and these are to be inspected in the spring of 1950 when their suitability will be assessed and a final selection made.

The work in the Cider and Fruit Products sections has continued to progress smoothly under the guidance of the Committee of the A.R.C. During the year it was possible to widen the scope of the experiments by the accommodation afforded by the provision of a small pre-fabricated building but the lack of proper facilities for comprehensive experiments on pilot-scale processing remains a serious handicap to progress.

During the year arrangements were made by the University for the inclusion of Mr. C. E. Hudson, Senior Advisory Officer, Ministry of Agriculture and Fisheries, on the Agricultural Committee and the Finance and Business sub-committee. Mr. Hudson will be warmly welcomed to these committees and his membership should provide a close link with the Ministry of Agriculture and the Horticultural Officers of the N.A.A.S.

In the 1948 Report details were given of a scholarship to commemorate the services to the Institute of Mr. John Wootton, Mr. Eldred Walker and Mr.

John Ettle. The first award was announced at the Annual General Meeting of members on July 21st, 1949. The recipient was Mr. A. P. R. Abbott who had been nominated by the Devon C.C. He began his six months' training in October and is obtaining experience both in cider making and cider orcharding. Mr. Abbott was the sole candidate for the 1949 award and it is hoped that in future years, when the advantages of the scheme become better known, the number of applicants will show considerable increase.

At the Annual Meeting it was decided further to commemorate the work of Messrs. Wootton, Walker and Ettle by the provision of a plaque to be hung in the Institute. A brass tablet mounted on oak bearing an appropriate inscription has been obtained from Messrs. Casswell and Grant, Taunton, and will be fixed in a suitable position in the library.

The first award of the Boots Scholarship was made in July to Miss Nancy F. Millis, M.Sc. (Agric.), Melbourne, Australia. The award is for a period of two years. Miss Millis graduated at Melbourne University after which she spent 2 years in the Bacteriology School of that University, working on denitrifying bacteria in soil. She was subsequently appointed to the Agriculture Department of External Territories, Commonwealth of Australia, as a District Agricultural Officer and was stationed in New Guinea. Her work at Long Ashton under Dr. Challinor is concerned with methods of isolation of bacteria from juices and ciders. These methods will later be utilised in a study of the prevalence in juices and ciders of bacteria which can give rise to disorders.

Staff.

Appointments. During the year it was possible to fill four vacancies on the scientific staff that had previously remained unfilled owing to the lack of candidates with suitable qualifications.

Mr. R. C. Codner was appointed as Microbiologist in the Cider Section from 1.10.49. Mr. Codner is a graduate in Botany of Bristol University and since leaving the University in 1947 he had been employed at the Glaxo Laboratories and later by the Distillers Co. Ltd., working on problems of streptomycin and fermentation.

Mr. F. W. Beech was appointed to the post of Biochemist in the Cider Section and took up his duties on 1.9.49. He received his undergraduate training in the Department of Chemistry at Birmingham University and obtained his degree in 1941. Since graduation Mr. Beech had served as a Flight Lieutenant in the Royal Air Force and after being demobilized held a post in a commercial cider firm.

Mr. J. D. Cuthbertson was appointed as Pomologist in the Pomology section to deal in particular with pomological problems of Cider fruits. He took up his duties on 1.9.49. Mr. Cuthbertson received his training in Horticulture at Wye College and graduated in the University of London in 1949. Prior to entering Wye, Mr. Cuthbertson had served in the army for 7 years and attained the rank of Major.

Mr. K. G. Stott was appointed to fill the post of Willow Officer which had been vacant since the retirement of Mr. H. P. Hutchinson in 1939. Mr. Stott commenced his duties on 12.9.49 but unfortunately he was called up for National Service in the Royal Air Force on 31.12.49 so that there will be a break in the work of the willow section until Mr. Stott's return in July 1951.

Mr. Stott received his scientific training in the Forestry School at the University College of North Wales, Bangor, and obtained his degree in the University of Wales in 1949.

Miss Lorna D. Kendall, a graduate in Botany in the University of Bristol, was appointed from 1.9.49 to replace Mrs. D. P. James in the Domestic Fruit and Vegetable Preservation Section.

Appointments to and promotions of assistant staff were as follows :

Miss Y. P. May, Analyst (Cider) from 1.1.49.

Mr. B. A. Notton, Research Assistant (A.R.C. Plant Nutrition Scheme) from 1.8.49.

Mr. A. J. Fisher, Analyst (A.R.C. Plant Nutrition Scheme) from 22.8.49.

Mr. A. H. Fielding, Research Assistant (Mycology) from 1.10.49.

Mr. D. G. Hallas, Research Assistant (Plant Nutrition), from 1.10.49.

Resignations. There were two resignations from the scientific staff during the year.

Mrs. D. P. James, who had been on the staff since 1st March, 1941, left on 31.1.49 to join her husband in Reading ; Miss Audrey Stone, who was appointed as chemist in the Fruit Products Section on 1.9.48, resigned to be married on 30.9.49.

The following members of the assistant staff resigned their posts during the year :

Miss M. D. Atherton, Assistant (Fruit and Vegetable Preservation) from 9.8.49.

Miss M. Anstey, Clerical Assistant, from 8.10.49.

Temporary Workers. The following have been accommodated in the Institute laboratories during the year :

Dr. H. Gleen of the A.R.C. team of workers on soil metabolism continued his work throughout the whole of the year.

Mrs. M. B. Tuke, member of the N.A.A.S., who came to the Institute on 13.12.48 to study sand culture techniques for bulbs, left on 30th September, 1949, and was succeeded by Miss P. Miles.

Mr. E. J. Winter, a member of the staff of the recently established National Vegetable Research Station, has been accommodated in the Biology building since 12th March, 1949, pending the building of laboratories at the N.V.R.S. Mr. Winter came to Long Ashton especially to study sand culture techniques and field experiment methods for horticultural crops. In addition he has pursued studies relating to lettuce culture.

As previously mentioned Miss N. F. Millis has worked from 12th September, 1949, as Boots Scholar, on the subject of " Methods of isolation and identification of micro organisms occurring in cider and apple juice."

Visits Abroad. The year has been noteworthy for some important visits abroad.

At the request of the Colonial Office, Dr. H. Martin visited Zanzibar and Pemba as a member of a scientific mission to decide methods to be adopted to check the spread of " Sudden Death Disease " of cloves which has devastated the clove Shambas of Zanzibar Island and now seriously threatens the plantations of Pemba. Dr. Martin was absent from the Institute on this work from March 9th-31st. The cause of Sudden Death is suspected to be an insect-transmitted virus and the treatment proposed is an eradication of diseased trees treated, before felling, with an insecticide to kill the insect vector. Six Long Ashton Dusters have been sent to Pemba for the latter purpose.

By agreement between the Dominion of Canada Scientific Service and the Ministry of Agriculture and the Agricultural Research Council, an arrangement for an exchange of workers was made with the Dominion Pathological Laboratory, St. Catherine's, Ontario, whereby Mr. Marsh was to work for a

year at St. Catherine's and Dr. R. S. Willison (Mr. Marsh's opposite number at St. Catherine's) to work at Long Ashton. The exchange came into force on March 1st, 1949.

Dr. Willison, who is a specialist in virus diseases of tree fruits, made a special study of virus problems in English orchards during his stay, working in collaboration with Mr. Crowdy and Dr. Luckwill at Long Ashton and workers at East Malling. During his stay in this country Dr. Willison carried out a survey of virus diseases in apple, cherry and plum orchards in Kent and the West Midlands, and completed a detailed anatomical study of plum trees infected with the recently recognised Stunt virus.

It was a real pleasure to the staff to have Dr. Willison with us and we hope that his experience at Long Ashton will prove valuable in his future work.

The Ministry of Agriculture, on the recommendation of the Agricultural Research Council, awarded Mr. Marsh a Travelling Research Fellowship for the study of research and development of fungicides in North America. With the kind co-operation of the Canadian Department of Agriculture and of university and other institutions in U.S.A., Mr. Marsh visited forty-one research centres in the major fruit-growing areas of Ontario, Nova Scotia, British Columbia, the Pacific States, the Lake States, the Shenandoah Valley and north-eastern U.S.A. Among the striking features noted in the American work on fungicidal plant protection were: (1) the attempts to control fungi on roots, (2) progress in the derivation of systemic fungicides, (3) the development of foliage sprays of high fungi-toxicity, (4) the use of spray concentrates.

Of the new protectant fungicides undergoing trials in fruit plantations, the three materials consistently showing exceptional control of the apple scab fungus were: dichloronaphthaquinone (Phygon), phenyl mercuric acetate (Tag HL/331) and a preparation of glyoxalidines (341 C). None of these is free from liability to cause spray damage in certain circumstances, and the naphthaquinone is a potential skin irritant. At present these newer fungicides are being recommended as occasional reinforcements of the standard American scab control programme of repeated applications of sulphur suspensions.

The effectiveness of concentrated fungicides dispersed in a high-velocity air-stream was seen to be highly dependent on weather conditions and exactitude of application. In fruit disease control this method has given its best results in the semi-arid areas of the West.

During his overseas visit, Mr. Marsh delivered twenty-two lectures and attended meetings of the American Phytopathological Society, the Canadian Phytopathological Society, the Agricultural Institute of Canada, New York State Insecticides and Fungicides Conference, the American Society of Horticultural Science, Niagara Peninsula Fruit Growers' Association, New York State Horticultural Society, etc. A full report has been made to the Ministry of Agriculture.

The Director was absent from the Station from July 8th to November 5th, on a visit to Australia and New Zealand. The main purpose of the visit was to attend, as a United Kingdom Delegate, the British Commonwealth Specialist Conference in Agriculture, convened to consider problems of Plant and Animal Nutrition in Relation to Soil and Climatic Factors. The actual conference occupied from August 22nd to September 15th. It comprised paper reading sessions in Adelaide from August 22nd-31st, a tour taking in centres between Adelaide and Canberra lasting from September 1st-12th and final plenary sessions for the formulation of resolutions in Canberra from September 12th-15th.

The conference was attended by representatives from all the Dominions, and the Colonies were represented by the Director of the East African Agriculture and Forestry Research Organization.

The gathering was unique in that it brought together for the first time workers in the various scientific subjects concerned with nutritional problems of crops and stock—plant and animal nutrition, soils and climatic factors. The papers contributed to the Conference, together with the discussions, sessional summaries and resolutions adopted are to be printed by H.M. Stationery Office.

As some time was available in Australia in addition to that occupied by the Conference, the opportunity was taken to visit some districts not covered by the Conference Tour. The following list of journeys made and of centres visited will indicate the variety of agricultural and horticultural problems seen or studied.

WEST AUSTRALIA. Fremantle, Perth; organised tour with officers of the Department of Agriculture of Western Australia along coast strip to south of Perth, thence inland to the east crossing belts of decreasing rainfall, then northward through dry areas and finally returning to Perth from the north east. On this tour various systems of husbandry were seen—forestry in the high rainfall Karri belt, dairying, citrus, apple and vine growing, market gardening, cereal growing and sheep raising for wool and mutton. Important mineral deficiency problems of crops and stock were demonstrated e.g. cobalt and copper of stock, and copper, zinc and phosphorus of crops.

From West Australia the journey was made to Adelaide over the Nullarbor Desert which provided a picture of the central arid region of salt bush and desert. The next stage of the journey east was made from Adelaide to Melbourne by air.

VICTORIA. From Melbourne a tour of the irrigated areas of the Goulburn and Murray valleys was made under the guidance of the State Horticultural Officer. The main areas visited were those around Shepparton and Mildura, the chief crops being citrus, grapes, pears, peaches and apricots. During this tour the main cultural problems of irrigation areas were demonstrated.

From Mildura the return journey was made by bus to Adelaide so as to see irrigated areas along the Murray valley in S. Australia.

Whilst in Adelaide visits were made to various research institutions under the University of Adelaide and the C.S.I.R.O., including the Waite Institute and the Research Institute for Animal Nutrition.

Under the guidance of the Chief Horticultural Officer for S. Australia visits were also made to local fruit growing centres, notably the Mount Lofty area of deciduous fruits—mainly cherries—and to the Barossa Valley, an important wine producing area.

Next followed the Conference tour from Adelaide. The first stage comprised the coastal area between Adelaide and Melbourne, including the important copper deficient area around Robe. Whilst at Melbourne the large city farm at Werribee was visited. This farm provides an example on a grand scale of the utilisation of town sewage for a farming project, the main activity being the rearing and fattening of cattle.

From Melbourne the route was northward, through Shepparton to the important sheep rearing area of the Riverina with an important C.S.I.R.O. research station at Deniliquin. After Deniliquin important irrigation areas in the Murrumbidgee basin were visited, a notable centre being Griffith, which is a centre of fruit growing—stone fruits and grapes and rice—and at which is

located a C.S.I.R.O. horticultural research station. From Griffith the route was in a south easterly direction through Wagga Wagga to Canberra. The area around Wagga is notable for erosion problems and a research station to study these problems has been established there. The country from Wagga to Canberra is mainly hilly grazing country forming part of the so-called Southern Tableland. In this area the famous Burrinjuck Dam, which is the main dam for the Murrumbidgee irrigation scheme, was inspected.

Canberra is the headquarters of the Divisions of Plant Industry and Entomology of the C.S.I.R.O., both of which were visited.

The remaining travel in Australia consisted of air flights from Canberra to Sydney and from Sydney to Perth. The latter journey provided an excellent opportunity of seeing Australian scenery on a grand scale, the journey over the Nullarbor desert being particularly instructive.

Special activities whilst in Australia were as follows :

Chairman of Conference Session Ba, " Effects of specific soil and climatic factors on the nutrition of plants "; broadcasts of summary of above session and on " Fruit Research in England " at Adelaide, and on " Some Impressions of fruit growing problems in Australian fruit centres " at Griffith ; address to the Commonwealth Club of Adelaide on August 30th, 1949, on " Mineral nutrition of crops in relation to agricultural production "; lecture to the Australian Institute of Agricultural Science at Melbourne on September 5th, 1949, on " Some aspects of the mineral nutrition of crops."

The New Zealand tour was made at the invitation of the New Zealand Council for Scientific and Industrial Research who also made a generous grant to meet expenses. The main centres visited were as follows :

Auckland. Plant Pathology laboratory ; Orotea Fruit Research Station ; orchards around Auckland—citrus and apples.

Christchurch. Lincoln College ; Agronomy and Wheat Research Institutes.

Nelson. Cawthron Institute ; Tobacco Research Station, Brooklyn ; farms in the Waimea and orchards in the Moutere Hills area, including the Appleby Research Orchard.

Wellington. Soil Bureau and Botany Division ; Headquarters of the D.S.I.R.

Palmerston North. Massey College ; D.S.I.R. Grassland Research Station.

Hamilton. Ruakura Research Station ; Soil Fertility Research Station ; farming areas between Hamilton and Rotorua.

The main points of special personal interest in the research projects were those relating to fruit and other horticultural crops, soil surveys and pasture problems. The achievements of the various research centres are impressive and it was of particular interest in connection with horticultural crops to note the similarity of problems in New Zealand and this country. This point is brought out strongly in comparing the research programmes of Long Ashton and the Cawthron Institute and the results obtained at the two stations.

The following lectures and talks were given during the tour :

Nelson. The 1949 Cawthron Lecture entitled " The mineral nutrition of crops, with special reference to fruit crops."

Christchurch. Agronomy Institute, talk on " Mineral nutrition of cereal crops."

Wellington. Lecture to Staffs of Soil Bureau and Botany Division on " Mineral nutrition of crops." Discussion with chief officers of D.S.I.R. and leading growers on " Crop nutrition problems."

Palmerston North. Massey College, Lecture to students on the "Mineral nutrition of crops."

In addition many discussions were held with groups of workers at the various centres on problems of mutual interest.

The tours both in Australia and New Zealand were very stimulating and provided experiences of a very wide range of problems. It was a particular pleasure to meet for the first time so many colleagues of the Commonwealth and to renew the acquaintance of many friends who have visited Long Ashton in the past. It was also pleasing to note the enthusiasm of these overseas workers and to gain first-hand information of their problems.

Finally grateful acknowledgment is due to the authorities in both Dominions for their generous help and hospitality, and for the excellent arrangements made to ensure the success of the visits.

Congresses. During the year a number of important congresses were attended by various members of staff, at which a number of papers were contributed from Long Ashton.

The most important were:

Second International Crop Protection Congress, London, July 20th-29th. At this congress Dr. Martin acted as Chairman of Section VI and Dr. Kearns as Secretary of Section V. Visits of congress members were made to Long Ashton on 28th and 29th July, 1949.

Biochemical Congress, Cambridge, August 19th-20th.

Symposium, Fermentation Industries, St. Andrews, July 23rd-30th.

Other Staff Items.

Mr. H. Gleen was awarded the Degree of Doctor of Philosophy in the University of Wales in June, 1949, for a thesis entitled "Some aspects of micro-biological sulphur metabolism in soil."

Mr. G. H. Jones was elected an Associate of the Royal Photographic Society in November, 1949, in recognition of the colour photography he has carried out at the Institute.

The assimilation of the staff into the new grading system for salaries was completed during the year by the assimilation of the administrative and clerical staffs.

The Annual Christmas Staff Party was held on December 21st, and was attended by approximately 200 members of staff and their friends. The staff was pleased to welcome on this occasion the Vice Chancellor and Lady Morris, the Bursar and Mrs. Singer, the University Auditor and Mrs. Maggs, and Mr. Woodall. The programme followed the usual pattern of competitive games, supper, an entertainment, consisting of variety items and two sketches contributed by members of the plantations and laboratory staffs, and dancing. The function was, as usual, a great success. It produced the usual number of surprise items and again demonstrated the theatrical talent which must surely only be kept dormant by the more serious purposes for which the Station exists.

Best thanks are due to all who contributed to a most enjoyable evening and, in particular, to those responsible for the entertainments and the catering arrangements.

Buildings and Land.

The structural work of the small building for the preparation of pots and sand for pot culture experiments was completed during the summer and the building has been in use for some portions of the work for which it is intended.

Certain fittings, however, are still incomplete but it is hoped that the building will be in full working order during 1950.

The plans for the special Pilot Factory building for the Cider and Fruit Products Sections have been completed and tenders have been obtained. The tenders have been forwarded to the Ministry of Agriculture for Treasury approval. Since it seemed unlikely that the building would be erected in the near future the Ministry and the Agricultural Research Council agreed to the erection of a small prefabricated building to enable a limited programme of small factory scale experiments to be carried out. Treasury approval to the scheme was obtained and a small Batley type building was erected and fitted for the work at a cost of £480. The building is in full use and the facilities have been greatly appreciated by the staff.

Two small temporary greenhouses have been sanctioned for special work on growth substances and radio active tracer elements. They will be ready for use in 1950.

Consideration has also been given to schemes for improving conditions in the large pot culture greenhouse. Since a large amount of the mineral nutrition work is now concerned with trace-elements, the earth floor of the house and the ash enclosure surrounding it have become unsatisfactory since they give rise to a considerable amount of dust. Plans have been approved for the fitting of a dust-free floor and the work is now in progress. The outside paint work of this building is still unsatisfactory and will require further treatment at an early date. The surrounds of the enclosure adjoining the building have had to be dismantled owing to the dilapidated condition of the main supports and the wire netting, and will require renewal after the enclosure has been re-surfaced. Meanwhile it is proposed to erect a temporary wire netting fence around the area.

The proposals regarding future building requirements at the Station have been considered by the Agricultural Research Council and the Ministry of Agriculture, and the Station has been asked to submit detailed plans, based on the proposals, to the Ministry for consideration by the Ministry and the Treasury. It is hoped that the plans will be ready for submission during 1950.

Lawns, with a few ornamental crab apple trees, have been established around the biology building in order to lessen the amount of dust, and to improve the appearance of the site.

The Station tennis courts were put into playing condition during the year and have been available for play during the summer. There is one hard court and one grass court, the former being laid by Messrs. En Tout Cas who also erected the wire surrounds to both courts. The courts have been much appreciated by the staff who have not had any facilities for recreation at the Station since 1939.

There is little change to be reported on the farm during the year, the most important being the completion of the clearance of Plot 18 of plum trees in preparation for the proposed cultural and manurial experiment on apples. The portion previously cleared was cropped with potatoes. Plot 16 has also been cleared.

Eight acres near the south boundary of the plantations, formerly rented by Mr. Butler, a neighbouring farmer, have been prepared for the planting of further bush cider variety trials in the winter of 1950-51. Ditches and drains have been cleared out and water clearance from the lower slopes has been improved.

In the nursery, rootstocks have been planted out for grafting with cider

varieties to provide trees for the proposed scheme of trial cider orchards. The first batch of trees should be ready for planting during the winter 1951/52.

Repair work has been continued on boundary and internal fences, in so far as supplies of materials for this purpose have allowed.

In spite of a dry season all newly planted trees and bushes made very good growth.

Crops were generally good, with little damage from frost, and the outstanding feature of the season was the heavy crop of black currants, 23 tons being harvested from 7 acres. The apple crop was a record for weight, size and quality.

As in previous years many requests for Long Ashton new varieties have been received, including apples, pears, plums and black currants, and propagating material was sent abroad to Denmark, Eire, France, Guernsey, Holland, Kenya, Norway, Spain, Sweden, Switzerland and U.S.A.

Research Activities.

Plant Nutrition. The principal studies on the nutrition of fruit trees concerned the correction of deficiencies of magnesium, copper and zinc, and the probable relationship of boron deficiency to bitter pit; comparison of cultural methods for apples; and methods of applying nutrient elements to trees, such as sub-soil placement, foliage and branch spraying, and stem injections.

In experiments on magnesium deficiency it has been confirmed that the quickest response to treatment results from 2% foliage sprays of magnesium sulphate applied at fortnightly intervals from petal fall. Both copper and zinc deficiencies have been proved in apples at Wisley and experiments have been extended to pears. From comparisons of various curative treatments—stem injections, dormant sprays and foliage sprays—it seems likely that the best treatment for commercial practice will be foliage sprays applied at petal fall. As these sprays may damage some varieties preliminary trials should be made prior to large scale treatment. Suitable sprays are Bordeaux 4:4:50 and zinc sulphate 2 lb. plus hydrated lime 1 lb. per 100 gall.

In the copper experiments varieties on M.XII rootstocks seem to be more susceptible to the deficiency than on M.II.

No results of boron treatment against bitter pit are yet available though it has been confirmed that the boron contents of many pitted fruits are quite high and would not be expected to respond to boron treatment.

Further results in cultural experiments on apples have confirmed the superior status of potassium and phosphorus in the leaves of trees under grass culture and the lower level of magnesium in the foliage of trees on M.I than of trees on seedling crab.

In comparisons of methods of applying nutrients, subsoil injection and foliage sprays of solutions of potassium sulphate gave better results than surface soil treatment. Subsoil injections of pseudo-solutions of ferri-silicate were inferior to solid stem injections of ferrous sulphate in curing a deficiency of iron in plum trees.

In the manurial experiments on blackcurrants it has been shown that under constant annual treatments the nitrogen status of the foliage has fallen steadily since 1947, indicating that as the bushes increase in age higher dressings of nitrogen are needed for maximum growth and fruit production.

Manurial experiments on vegetable crops carried out during 1949 were seriously affected by drought, which affected germination and restricted growth.

Investigations on the mineral nutrition of farm crops were mainly in continuation of work carried out in 1948. Several important results were obtained in experiments with molybdenum. In the field it was possible to show striking results from molybdate sprays and from liming on "whiptail" which was very severe in commercial holdings under the dry conditions obtaining during the season. In these experiments it has been shown that deficiency levels of molybdenum in the leaves of cauliflower and broccoli plants are of the order of 0.02 to 0.05 p.p.m. whilst in healthy plants they are usually above 0.3 p.p.m.

In sand culture experiments a survey of the effects of molybdenum deficiency on a wide range of Brassica crops was made and the inter-relationships of molybdenum and manganese examined in view of the special relations of these elements in crop problems on acid soils. The relationships do not appear to be simple though, on the whole, high molybdenum treatment appeared to accentuate the toxic effects of high manganese. The molybdenum contents of a range of crops receiving normal and deficiency levels were also studied. The values vary considerably for different crops. It was established that cauliflowers given excess molybdenum can accumulate high concentrations of the element—old leaves contained >400 p.p.m. and young leaves >350 p.p.m.

Further experiments were carried out on iron nutrition, including the inter-relationships of iron, potassium and phosphorus in potato, and the effects of heavy metals on iron metabolism in the presence of nitrate or urea as sources of nitrogen.

In the Fe/P/K experiments iron deficiency was accentuated by high phosphorus levels and vice versa. A similar reciprocal relationship was shown by potassium and phosphorus. Potassium deficiency effects were delayed by high iron levels but ultimately became very severe. The beneficial effects of high potassium when iron is low were confirmed. The highest yields of whole plants (dry matter basis) were obtained with low iron and high potassium, high phosphorus levels, but the highest yields of tubers occurred with medium iron combined with high potassium and high phosphorus.

Tubers from low phosphorus, high potassium treatments gave a high proportion of tubers affected with a non-pathogenic medullary necrosis.

The results obtained in the heavy metal/iron experiments generally confirmed those given in the 1948 report. Molybdenum added to an excess of a heavy metal element usually accentuated the iron deficiency effects. Results showed some variation between the urea and nitrate series but there was no clear cut difference as regards the effects on chlorosis.

In experiments on the soil acidity complex, potato, dwarf bean and swede were grown at two levels of phosphorus and manganese, and three levels of aluminium. The order of sensitivity of the crops to aluminium excess was potato $>$ dwarf bean $>$ swede and the reverse relationship obtained for excess manganese. Excess manganese effects were usually increased by excess aluminium whilst an excess of phosphorus reduced the toxic effects of aluminium. Excess aluminium alone did not reduce the yield of swedes.

In developments of methods for experiments on trace elements in sand cultures *Aspergillus niger* has been used successfully for checking the purity of nutrient reagents, particularly for molybdenum.

In laboratory investigations on diagnostic methods for mineral nutrients, further progress has been made in developing procedures for tissue tests. The use of various extractant procedures with different macerators has been examined and new colour tests for some of the nutrients have been tried.

Preliminary work has also been carried out to test the applicability of chromatographic methods for tissue test purposes.

The use of *Aspergillus niger* as a test organism for the determination of micro nutrients in soils has been further investigated and tentative standards have been adopted for Mg, Cu, Zn, Mn and Mo. The method has been applied to instances of deficiencies of Cu, Zn and Mo in crops during 1949. For a soil at Wisley producing deficiencies of Cu and Zn in apple trees values of 0.2 μg . Cu and 1-2 μg . Zn were obtained as against 20 to 30 μg . Cu and $> 10 \mu\text{g}$. Zn for healthy soils. For soil tests for molybdenum availability as little as 0.1 gm. soil is necessary and the pH of the culture medium must be between 2-3 pH. The *Aspergillus* method has also been successfully used for the bioassay of molybdenum in ciders.

Experiments with radio active phosphorus P^{32} have been pursued on crops and on *Aspergillus*, in the latter instance to see whether the method is likely to be successfully applied to nutritional problems of micro organisms. With *Aspergillus* radiation injury occurred from dosages of 50 microcuries.

Field experiments to determine the relationships between the level of manganese in various plant organs and crop yields were carried out on oats and sugar beet. The chemical determinations are still incomplete but parallel trends between "tissue test" manganese in leaves and yields are evident.

In these experiments foliage sprays proved to be the most effective method of applying manganese whilst sulphur dressings at 2 cwt. per acre applied with the seed were ineffective.

Experiments on the use of chromatographic methods for the determination of organic constituents in plants were continued on apple and pear tissues, particularly leaf, bark and fruit. Various solvents for the irrigation of the paper chromatograms were studied; phenol acetic acid and butanol-acetic acid mixtures have been found generally useful for phenolic substances and alkaline phenol and collidine are preferred for sugars. The examination of colour reagents for showing the positions of phenolic reducing substances on the chromatograms has shown the outstanding value of ammoniacal silver nitrate.

Among the substances identified in apple tissues the glucoside phloridzin has been found in all parts with the exception of the flesh of the fruit. This substance has not been detected in any part of the pear, which is contrary to statements in the literature. Arbutin in the pear seems to correspond to phloridzin in the apple.

It has been shown that the nature of the tannins in the different parts of trees differ and that so called "tannin fractions" are likely to be mixtures.

Examination of the amino acid contents of plants grown under conditions of deficiencies of molybdenum and manganese in sand culture and of the latter in field crops have confirmed previous observations that molybdenum deficiency is associated with low amounts of amino acids and manganese deficiency with high amounts. Moreover, some amino acids are notably affected, e.g. glutamic acid and its amide glutamine by a deficiency of molybdenum and arginine and asparagine by manganese deficiency.

The application of chromatographic methods to ciders and apple juices promises to be of value for the identification of tannins and sugars.

Field studies of acid soils by means of indicator crops were continued. The problems on the plots include deficiencies of P, Mg, N, Mo and B, and excess of Al and Mn.

During the year the scheme for reporting micro nutrient disorders in crops in England and Wales, which is sponsored by the Ministry of Agriculture

and the Agricultural Research Council, was inaugurated. The disorders are reported by Advisory Chemists in the N.A.A.S. to Long Ashton where records are kept.

In the investigations on soil metabolism a study has been made of thiocyanate metabolism on nitrification. It has been shown that whilst concentrations of sodium thiocyanate of the order of M/140 inhibit the nitrification of ammonium sulphate, the thiocyanate is eventually metabolised to ammonium sulphate after which nitrification proceeds. Respiration studies have been carried out on soils enriched with nitrobacter and with thiocyanate oxidising bacteria.

An attempt has been made to use the soil perfusion method to determine the factors affecting the availability of molybdenum in soils. Solubility of molybdenum in the perfusate was probably influenced by pH but no evidence was obtained to show that soil organisms affect availability.

Pomology. In the pomology section work was continued on the action of growth regulating substances on fruit development and fruit drop. Detailed studies have been made of the hormone in apple seeds associated with the development of the fruit. The concentrate found in the seeds of the variety *Crawley Beauty* was comparatively small but the peak of concentration occurred normally.

Attention has been given to an inhibitor which accumulates in the outer parts of the seed late in the season. This inhibitor, like the growth substance, is acidic in character. The effects of various synthetic growth substances, used alone and with carbohydrates, on fruit bud differentiation, are being examined.

Good separations of indolyl-, naphthyl- and phenoxy-growth substances have been obtained by the method of paper-partition chromatography. The method is to be applied to the separation and identification of naturally occurring hormones.

Routine bioassays have been carried out to assess the value of various substances as growth promoters and inhibitors. A number of substituted aryl phthalamic acids show promise as growth regulators.

Detailed studies of the vegetative characters of cider varieties have been begun with a view to using these for identification purposes.

The blossoming periods of the varieties are also being recorded and experiments are in progress to establish self and intercompatibility.

Pests and Diseases and Their Control. A bud-burst application of an emulsified DDT spray has been shown to provide an effective substitute for the standard winter wash treatment with DNC petroleum. Wettable DDT powders were less satisfactory. There was no evidence that red spider infestation was encouraged by this DDT treatment.

Investigations have been continued on the absorption, translocation and phytotoxicity of systemic phosphorus insecticides applied to foliage, or to the soil in which the plants are growing. Soil application gives the more lasting systemic action but, if the insecticide is sufficiently non-volatile, leaf application was found to give an eight weeks' protection of apple seedlings from aphid attack. The contact toxicity of likely constituents of commercial Parathion is under examination. Parathion has been found, on heat treatment, to undergo isomerization to form less insecticidal but more poisonous compounds.

Work has continued on the synthesis and insecticidal bioassay of compounds isosteric with DDT and BHC. A critical examination of other chlorinated hydrocarbons showed that 2:6:7-trichloro camphene and two chlorinated

naphthalene derivatives were of different mode of action to DDT ; a chlorinated dicyclopentadiene from British sources was inactive.

Laboratory and field bioassays of fungicides have been continued, using apple scab, pear scab and brown rot fungi as test organisms. Winter sprays with phenyl mercury chloride have greatly reduced the inoculum of brown rot on mummied fruits, but the reduction has not been sufficient to arrest the disease on the succeeding crop. On pears, a glyoxalidine spray controlled scab on Doyenne du Comice without causing damage, but on apples this spray caused some reduction in yield of Cox's Orange Pippin.

In investigations of tree fruit virus diseases, it was found that apparently healthy Lord Lambourne scions budded on to certain Malling stocks developed rubbery wood. Anatomical studies of Plum Stunt have been made, and preliminary indexing trials carried out.

A labour-saving system of automatic spraying has been developed, using nozzles carried on a mast erected on a mobile sprayer. An air-blast machine capable of applying either concentrates or dilute washes has been constructed and a power duster has been designed for tropical use.

Cider. In cider investigations the effect of stage of maturity of the fruit at the time of milling and pressing was studied. The treatments did not effect significant changes in the vintage qualities of the resultant ciders ; specific gravity showed little change but there was a general decrease in acidity with increasing maturity.

Fermentation studies have included the use of pure and natural yeasts. The former were selected after preliminary trials with 81 yeasts. The role of various nitrogenous constituents is under investigation. In particular "total nitrogen" and soluble nitrogen have been followed during fermentation.

An examination of the acids in various ciders has shown the presence of a large proportion of lactic acid in the varieties Sweet Alford and Michelin, both of which are low acid sorts.

Investigations on the "cider sickness" bacteria have been continued. Special attention has been given to the nutrient conditions favouring the growth of the organisms. The content of molybdenum found in 23 ciders ranged from 0.015 to 0.0007 p.p.m. The Mo content of the fruit was increased by injecting branches with molybdates.

Fruit Juices. The materials for apple juice manufacture have been examined in relation to the variability of fruits from individual trees and the changes occurring in the composition of the fruits during storage.

Experiments were continued on methods of stabilising juices and on the effects of de-acidification methods. The efficiency of a number of resins as ion exchangers has been determined and the possibility of the use of the ion exchange method for commercial and research purposes has been considered.

In connexion with problems of clarification the effects of various enzyme preparations have been determined and the activity of pectase in some apple varieties has been examined. It has been shown that the juice pectase possesses definite activity and may help in the clarification of the juice.

The effects of varying levels of nitrogenous manuring of blackcurrants on the ascorbic acid content of juices and syrups were determined.

The results obtained were similar to those previously reported for the fresh fruits ; high nitrogen contents were associated with low amounts of ascorbic acid and *vice versa*. The manurial treatments did not influence ease of juice extraction nor the juice values for specific gravity, sugar, acid, pectin and tannins.

Fruit and Vegetable Preservation. The domestic preservation of fruit and vegetables section continued its investigations on losses of ascorbic acid from products arising from processing methods and the use of various containers and covering materials. The use of SO_2 for fruit preservation did not affect the ascorbic acid content.

Microbiological assays of the Vitamins of the B_2 group were continued for various fruits and vegetables.

Heat penetration tests made during the processing of fruits and vegetables have shown the need for revising some of the recommended methods. Investigations on the browning of processed pears, plums and apples have demonstrated that temperatures ranging from 160° to 185°F are necessary to inactivate the enzymes concerned.

Other Activities.

Members' Day. The annual field day for members was held on June 21st, in combination with the Annual Meetings of the Governors and Members of the National Fruit and Cider Institute.

A varied programme illustrating important investigations in progress and practical points of management had been prepared for the occasion. The main items were as follows :

1. Field plots—tree staking and protection ; pruning of young cordons, dwarf pyramids, permanent bush and filler trees ; management of bush cider orchards ; blackcurrant cultivation.

2. Pot culture exhibits—problems of acid soils ; iron relationships in plant nutrition ; methods for investigating problems of crop nutrition.

3. Cider making exhibits illustrating : apple pomace as a source of pectin ; distribution of cider orchards in the West of England. The recommended list of varieties for planting was also displayed.

4. Main laboratory—rapid chemical tests on plant tissues for detecting mineral deficiencies ; microbiological assay method for determining the availability of micro nutrients in soils ; use of ion exchange methods in fruit products research ; methods of preserving fruits and vegetables, and tests for assessing the efficiency of methods ; composition of fresh and processed fruits.

5. Biology laboratory—demonstrations of the control of pests and diseases and of the use of systemic insecticides.

6. Machinery—exhibit of spray nozzles and field demonstration of the application of Aerosols.

Cider Sampling Day. The annual sampling day was held on May 5th. The function took a similar form to those held in the post-war years. The main subjects demonstrated were the effects of pasteurisation on the flavour of different types of cider, blending in relation to the development of cider sickness, and the qualities of varieties recommended for planting.

It was shown that the effect of pasteurisation on flavour could be detected, but that such effect was slight when the process was properly carried out. The factor of acidity was considered in relation to pasteurisation : blends of a selected cider with varying proportions of a high acid cider were tasted in the pasteurised and unpasteurised states. The general opinion, as shown by voting, was that the high acid ciders were less affected by pasteurisation than the ciders of lower acidity—a somewhat unexpected result.

Blending times in relation to the resistance of the ciders used in the blend on the development of cider sickness were considered and the importance of blending early was shown.

Attention was also given to single-variety ciders made from the varieties recommended for future propagation by the Cider Advisory Committee and to ciders made from varieties submitted for Vintage Quality Trial.

In addition to the ciders two single variety perries and a number of unfermented apple juices were tasted. The latter comprised juices prepared from Bramley's Seedling by four different methods.

Exhibits and charts relating to cider fruit propagation, blossoming dates of recommended varieties, crop records (from bush and standard orchards) were also shown.

During the afternoon the trial cider bush plots and orchards were inspected and demonstrations of spraying and orchard management given.

Visitors. Fifty-three organised parties visited the Station, which was the maximum number that could be received. The parties included two groups of N.A.A.S. Horticultural officers, who attended for two-day courses, a scientific mission from Canada, two parties from the 2nd International Congress of Crop Protection, parties of French and Australian farmers, group of French, German, Norwegian and Swiss University Students, Danish Horticultural Advisers and Extension Officers, and a party of Danish Young Farmers. The full list is as follows :

Association of Scientific Workers, Wells Branch.
 Australian Farmers.
 Bath Young Farmers' Club.
 Bourton and District Horticultural Society.
 Bristol Boy Scouts.
 Bristol Central Brotherhood and Sisterhood.
 Bristol East Conservative and Unionist Association.
 Bristol Folk House Students.
 Bristol Grammar School Scientific Society.
 Bristol International Club.
 Bristol Teachers Nature Group.
 Bristol Tramways and Carriage Company.
 Bristol University Employees' Association.
 Canadian High Level Mission.
 Cardiff and District Section of the Royal Institute of Chemistry.
 Clifton High School Sixth Form Scientists.
 Congresbury Garden Club.
 Danish Horticultural Advisers and Extension Officers.
 Danish Young Farmers.
 French Farmers.
 French, German, Norwegian and Swiss University Students.
 Gloucestershire Home Food Production Committee.
 Hereford Teachers' Training College.
 Horticultural Education Association, South Western Branch.
 Institutional Management Association, S.W. Branch.
 Second International Congress of Crop Protection, Section V.
 Second International Congress of Crop Protection, Section VI.
 Kemp-Welch Secondary School, Parkstone, Dorset.
 Lackham School of Agriculture, Lacock, Wiltshire.
 Melksham and District Young Farmers' Club.
 Nailsea Produce Association.
 National Agricultural Advisory Service Crops and Grassland Officers.
 National Agricultural Advisory Service Horticulture Officers—Refresher Course.
 National Agricultural Advisory Service Horticulture Officers—2-day Course.
 National Farmers' Union, Devon Horticultural Section.
 Oakley Teachers' Training College for Men, Cheltenham.
 Picklenash School, Newent, Glos.
 Reading University Students.
 Royal Agricultural College, Cirencester.
 Research Chemists (Insecticides).
 Seale Hayne Agricultural College, Newton Abbot, Devon.
 Somerset Beekeepers' Association.
 Somerset Farm Institute, Cannington, Nr. Bridgwater.
 Somerset Federation of Women's Institutes Produce Guild.
 Somerset Fruit Growers' Club.

Waterperry Horticultural College.
 Wells Natural History and Archaeological Society.
 Weston-super-Mare Grammar School for Girls.
 Whitelands College, Putney.
 Wiltshire Federation of Women's Institutes Produce Guild.
 Wiltshire Growers.
 Wiltshire Teachers.
 Women's Farm and Garden Association, S.W. Group.

Many scientists from overseas countries visited the Station for both long and short periods. Among these the following may be specially mentioned: Mr. R. S. Beckwith, of the Division of Soils, C.S.I.R.O., Waite Institute, Adelaide, Professor G. W. Leeper, of the Department of Agricultural Chemistry, Melbourne University; Mr. A. E. Martin, Division of Soils, C.S.I.R.O., Mr. H. R. Powell, Senior Technical Officer of the Agricultural Chemicals and Pharmaceuticals Division of I.C.I., Mr. C. W. Strutt, Overseas Adviser and Mr. R. F. Williams, Plant Physiologist of the Irrigation Research Station, Griffith, N.S.W., all from Australia; Dr. G. H. Berkeley, Director of the St. Catharines Laboratory of Plant Pathology, Ontario, Mr. W. F. Darke, of Ottawa, and Dr. J. T. W. Spinks, Head of the Department of Chemistry, Saskatchewan University, came from Canada; Mr. H. H. Croucher, Director of Agriculture, British Guiana; Mr. E. A. Evans, of the United Africa Co., Congo; Miss Johanne Jorgensen, A/S for Kemisk Industri, Denmark; Mr. Khalifa, of the University of Cairo, Egypt; Mr. M. Cochois, of the French Embassy, Mr. H. Darpoux, of Station Pathologie Vegetale, INRA, Versailles, Mr. H. Heslot, of the Institut Nationale Agronomique, Paris, Mr. A. Reifenberg, President du Graf, Paris, and Mr. G. Viel, Laboratoire Phytopharmacie, Versailles, all from France; Mr. R. A. Webb, of the Human Nutrition Research Unit of the Medical Research Council, Gambia; Mr. W. A. Hughes, of the Gold Coast; Dr. Deist, of Wageningen, Dr. P. R. den Dulk, of the Soil Advisory Service, Bennekom, Dr. J. J. Lehr and Mr. J. M. Wybenga, of the Nitrate Corporation of Chile, Ltd., Professor L. Seekles, of the Veterinary Chemistry Department of Utrecht University, Mr. J. Wind, Director of the Central Institute of Agricultural Research, Wageningen, Dr. G. C. Redlich, of Nederlandsch Landbouw Kalk Bureau, Zwolle, Messrs. Stadhouders and Vrijhof, Horticultural Advisory Officers of the Royal Netherlands Ministry of Agriculture, Dr. van der Paauw, of the Soil Institute, Groningen, and Mr. A. W. van der Plassche, Director of Horticulture of the Royal Netherlands Ministry of Agriculture, all from Holland; Mr. Somana, of the Consolidated Coffee Estates, Ltd., Pollibetta, India; Mr. R. F. Innes, of the Research Department of the Sugar Manufacturers' Association, Jamaica; Lt. Col. R. O. Miller and Colonel Ashford, of Jersey; Mr. K. Thorpe, Senior Research Officer of the Department of Agriculture, Kenya; Mr. Ley Teik Gunn, of the Department of Agriculture, Federation of Malaya; Dr. B. W. Doak, of the D.S.I.R. Plant Chemical Laboratory, Mr. F. J. T. Grigg, Director of the Dominion Laboratory, Wellington, Mr. Hendrie, Overseas Adviser, Mr. H. Jacks, of the Plant Diseases Division, Auckland, and Mr. K. M. Williams, of the D.S.I.R., Wellington, all from New Zealand; Mr. C. A. Thorold, of the Department of Agriculture, Nigeria; Miss Marit Eggen Ogrim, of Oslo University, Norway; Mr. James Gordon, Agricultural Officer of North Borneo; Dr. N. Z. Viljoen, of the Potchefstroom College of Agriculture, Orange Free State; Dr. R. Cabral, Director of the Vegetable Laboratory, Lisbon, Portugal; Dr. R. I. Nel, Director of the Western Province Fruit Research Station, Stellenbosch, S. Africa; Dr. Werner Schwarzenbach, of the Wädenswil Fruit Research Station, Switzerland; Mr. F. R. Sanders, Department of Agriculture, Tanganyika; Dr. H. H. Evans

and Mr. Paul Holliday, of the Imperial College of Tropical Agriculture, and Mr. R. H. Forster, all of Trinidad ; Mr. Nasoyhi Bey Tahir, Deputy Minister of Agriculture, Transjordan ; Dr. J. G. Horsfall, of the Connecticut Agricultural Experiment Station, and Drs. C. Lalor-Burdick and A. G. Norman, all of U.S.A.

Members of staff were glad of the opportunity to meet these colleagues from overseas and to discuss with them problems of mutual interest.

Shows and Demonstrations. Exhibits were staged at the following shows :

Bath and West Show—Ashton Park, Bristol, May 1st to 4th.

Royal Horticultural Society—Chelsea Show, May 24th to 27th.

Three Counties Show—Worcester, June 14th to 16th.

Bristol Horticultural Show—Bristol, August 31st to September 2nd.

Royal Horticultural Society—London. Fortnightly shows. Fruit Group Exhibits. September 4th, 5th, November 29th, 30th.

At the Bath and West Show, in addition to a general exhibit, special cider making demonstrations were given daily by the Staff of the Cider Section.

Students and Voluntary Workers. The number of research students still remains strictly limited because of the lack of bench space. Mr. R. J. W. Byrde has continued to work at Long Ashton under Mr. Marsh by arrangement with Prof. R. H. Stoughton and Reading University. Mr. Byrde has continued his investigations of Brown Rot of Apple and Plum. Mr. E. J. Skerrett, A.R.I.C., who is a student of the University, registered for the degree of M.Sc. in Organic Chemistry, is working under Dr. Woodcock on the problem of the relationship between chemical constitution and insecticidal properties.

Mr. John Weston, B.Sc., a student at University College, Aberystwyth, and Mr. G. T. Ball, an undergraduate of the University of Bristol, both worked during August and September under Dr. Nicholas, studying problems of plant biochemistry. Mr. S. T. C. Wright, an undergraduate in the University of Reading, worked in the Pomology section under Dr. Luckwill in July and August.

The following courses were given in the Domestic Fruit and Vegetable Preservation Section.

Preservation and Judging Preserves. July 4th–22nd.

Preservation of Fruit and Vegetables. August 8th–19th.

Preservation of Fruit and Vegetables. August 22nd–September 2nd.

Judging Preserves. September 5th–8th.

For assistance given in connection with these courses, thanks are due to Miss M. Bowen, who attended during term-time with the kind permission of the Principal of the Gloucestershire Training College of Domestic Science, and to Miss D. L. Simpkins, of Bath.

Mr. J. F. Bevis has again helped in the Plantations and with experimental plots ; our sincere thanks are due to him for his valuable assistance.

Acknowledgments. Several members of the senior staff have again undertaken special duties in addition to their normal work. Dr. Martin has continued to give valuable help with the research programme and in advising on special equipment and apparatus. During the Director's absence in Australia and New Zealand he deputised for the Director on all scientific and academic matters and together with Mr. Leggett and Miss Evans carried on the routine business of the Station. Dr. Kearns has continued to give valuable assistance in connection with building problems and in organising and supervising repair work to equipment, machinery and tools for the laboratories and farm. Under his guidance many improvements have been effected to buildings and machines. Mr. Spinks continued to act as librarian and, during the absence of Mr. Marsh

in Canada, he also dealt with editorial matters, visitors and parties. Dr. Woodcock continued in the office of Chairman of the Welfare Committee in which capacity he was responsible for the organisation of the Christmas Social. Miss Sturdy acted as Chairman of the Canteen Committee, a position entailing much day to day work. To these and to other members of staff who have helped in other ways, best thanks are due.

Grateful acknowledgment is also made to all members of the assistant and technical staffs and to all employees for their loyal support in carrying out the research programme.

Finally, thanks are due to all growers and commercial firms who have co-operated in experiments or helped in other ways.

THE PRODUCTION OF CIDER FRUIT ON BUSH TREES. OBSERVATIONS ON YIELDS, 1945—1949.

By J. D. CUTHBERTSON and RITA M. STICKLEY.

INTRODUCTION.

Previous progress reports on the trial bush cider plots, established in the autumn of 1935, have appeared in the Annual Reports for 1941 (1), 1942 (2) and 1947 (3). In this trial a number of two acre plots were established at widely separated centres outside the Research Station. An eight acre plot was also laid down at the Research Station. This plot at Long Ashton has been given more specialised treatment than the remainder and therefore it is considered separately in this report. Data have been received from seven of the outside centres.

A. TRIALS AT COUNTY CENTRES.

The seven centres are Kings Acre, Hereford; Much Marcle (Herefordshire); Newnham Bridge (Worcestershire); Poundisford (Somerset); Whimple (Devonshire); Compton Greenfield (Gloucestershire); and Faversham (Kent). The last report on the County Centres, which was made in 1947 (3), gave details of relative yields for the years 1941-7.

Methods of Assessment of Yield.

Actual crop weights for each variety have been obtained from Hereford (1947-9), Faversham (1947-9), Much Marcle (1948) and Poundisford (1948). The maximum number of trees of the same variety at any one centre is in nearly all cases ten. Yields have been calculated on the basis of 108 trees per acre (planting distance 20 ft $\times$ 20 ft.) on all plots excepting that at Hereford. On this latter plot the planting rate was 160 trees to the acre and calculations have been made on this basis; these results are therefore not directly comparable with those obtained from the plots with the wider planting.

For the remaining data reliance has had to be placed on a visual crop estimate made in September or October 1948-9. This estimate has been expressed in terms of a mean relative yield figure which varies from 0 to 10, the latter figure representing a full crop. Reference should be made to the report by Stanton (3) for a complete table of the corresponding values for mean relative yield figures.

Half the trees have been worked on Seedling Crab stock and half on Malling Type I, but any differences in cropping which may be due to this factor, have been neglected.

Yields: Hereford and Faversham 1947—9.

Table IA and Table IB show individual crop weights for the years 1947 to 1949 at the Hereford and Faversham centres respectively. The plot at Hereford was grassed down in the early years of the war while that at Faversham has been maintained under arable conditions since planting.

In general the yield in 1948 was less than that for either 1947 or 1949. At Hereford, White Close Pippin, White Jersey and Medaille d'Or II, and at Faversham, Chisel Jersey, show a reverse trend.

TABLE IA.
CROP YIELDS OVER A PERIOD OF THREE YEARS: TONS PER ACRE (CALCULATED)
HEREFORD.

Variety	Seasons after planting			Total Yield	Average Yield Per Year.
	12	13	14		
Variety	1947	1948	1949	1947-9	1947-9
Bulmer's Norman ..	16.0	1.0	14.0	31.0	10.3
Yarlington Mill ..	12.0	0.5	15.6	28.1	9.4
Michelin	12.0	2.1	13.8	27.9	9.3
Woodbine*	8.8	1.0	13.2	23.0	7.7
Dabinett	11.2	1.8	9.0	22.0	7.3
Ellis Bitter	1.4	5.3	15.2	21.9	7.3
White Close Pippin*	5.2	9.1	6.0	20.3	6.8
White Jersey* ..	3.8	9.8	6.7	20.3	6.8
Sweet Alford ..	4.8	1.8	8.6	15.2	5.1
Reine des Pommes	7.4	1.4	6.4	15.2	5.1
Red Jersey*	4.2	2.5	6.4	13.1	4.4
Sherrington Norman ..	Nil	3.5	6.6	10.1	3.4
Tardive Forestier ..	7.0	0.2	2.4	9.6	3.2
Medaille d'Or II ..	0.3	5.6	Nil	5.9	2.0
Silver Cup	1.2	Nil	3.4	4.6	1.5
Knotted Kernel ..	2.4	Nil	2.0	4.4	1.5
Foxwhelp	3.4	Nil	0.8	4.2	1.4
Kingston Black ..	0.2	0.2	2.4	2.8	0.9
Royal Wilding ..	0.3	Nil	0.8	1.1	0.4
Average Yield Per Year—All Varieties	5.3	2.4	7.0	—	—

* Not represented at Faversham.

The heavy crop which was borne in many areas in 1949 is reflected in the high average yield figure for all varieties for that year.

It is not possible to relate this seasonal fluctuation in yield to any specific external factor.

Comparison of Varieties at the Two Centres. Accurate comparisons of the varieties at the two centres are not possible owing to the different planting distances, but the following points are of interest.

Of the varieties appearing in both Tables IA and IB, Bulmer's Norman, Michelin and Yarlington Mill are recognised as exceptionally heavy croppers making fairly large trees and bearing medium to large fruits. Reine des

TABLE 1B.
CROP YIELDS OVER A PERIOD OF THREE YEARS: TONS PER ACRE (CALCULATED).
FAVERSHAM

Variety	Seasons after planting			Total Yield	Average Yield
	12	13	14		
	1947	1948	1949	1947-9	1947-9
Bulmer's Norman ..	15.8	6.3	19.2	41.3	13.8
Michelin†	15.4	1.5	23.7	40.6	13.5
Perthyre†	11.9	7.2	18.8	37.9	12.6
Reine des Pommes ..	15.4	5.3	16.2	36.9	12.3
Sherrington Norman ..	13.9	3.2	19.6	36.7	12.2
Ellis Bitter	5.6	4.3	23.6	33.5	11.2
Broadleaf Norman†	11.8	5.6	16.0	33.4	11.1
Yarlington Mill ..	12.7	2.5	18.2	33.4	11.1
Tardive Forestier ..	16.2	Nil	14.3	30.5	10.2
Chisel Jersey* ..	6.6	13.5	7.8	27.9	9.3
Medaille d'Or II ..	11.2	1.7	15.0	27.9	9.3
Royal Wilding ..	10.2	1.5	11.9	23.6	7.9
Silver Cup	6.0	1.9	13.9	21.8	7.3
Kingston Black ..	6.5	0.5	11.2	18.2	6.1
Knotted Kernel ..	6.8	0.8	9.0	16.6	5.5
Sweet Alford	4.0	4.9	4.3	13.2	4.4
Dabinett	4.5	1.6	6.1	12.2	4.1
Foxwhelp	4.5	0.1	4.2	8.8	2.9
Average Yield Per Year—All Varieties	9.9	3.5	14.0	—	—

* Not represented at Hereford.

† Headworked at Hereford in 1948 as unproductive.

‡ Only 2 trees.

Pommés has smaller fruits but is cropping well at both centres. Ellis Bitter, although giving a fairly high average yield due to a very good crop in 1949, has not cropped consistently well over the three years.

Dabinett is a variety which is usually considered to be a heavy cropper, but it makes a small tree and thus crop weight per acre tends to be low when it is planted at a distance suitable for more vigorous varieties.

Yields : Other County Centres. 1948—9.

It is difficult to make reliable comparisons between the remaining county centres as actual crop weights were only obtained from two, namely Much

Marcle and Poundisford, in 1948. The crop at Much Marcle in 1948 was good, Bulmer's Norman, Reinette Obry and Slack-ma-girdle yielding at a calculated rate of just over 10 tons per acre each. Yarlington Mill gave a yield of 7.7 tons per acre and Sweet Alford 6.2 tons per acre on this plot. At Poundisford Sweet Alford cropped at 7.6 tons per acre, but in general yields here were not good.

The varieties Fair Maid of Devon, Star of Devon, Tremlett's Bitter, Yarlington Mill and Dabinett all carried full crops at Whimple in this same year. Other varieties which cropped almost as well (mean relative yield 9.0—8.0) were White Close Pippin, Medaille d'Or II, Bulmer's Norman, Frederick and Sweet Alford. Yields on the plots at Newnham Bridge and Compton Greenfield in 1948 were not outstanding.

In 1949 the crops carried on the plots at Newnham Bridge, Whimple and Compton Greenfield were above average. Varieties which cropped well were Reinette Obry, Dabinett, Yarlington Mill, Reine des Pommes and Bulmer's Norman (mean relative yields 7.6—5.6). Vintage varieties such as Frederick, Knotted Kernel and Silver Cup carried lighter crops with a mean relative yield from 3.3 to 2.3. Ellis Bitter at Newnham Bridge and Yarlington Mill at Compton Greenfield both carried full crops.

At Poundisford the crop was poor and at Much Marcle it was almost negligible.

Discussion.

Important factors affecting yield appear to be soil type, local climatic conditions and standard of management. At Much Marcle the plot is sited on a fine sandy silty loam of shallow depth and the negligible crop obtained there in 1949 can be attributed to drought conditions which were brought on by the exceptionally dry summer of that year. This plot was grassed down in 1948 and it is possible that the check to cropping experienced in 1949 was accentuated by this factor.

At Poundisford, trees are showing symptoms of severe nitrogen starvation and this has adversely affected the yield.

During the war the plot at Whimple carried several straw crops and the condition of the trees suffered in consequence. More recently the plot has been under a cover crop of white clover and chicory and the nitrogen status of the soil has been built up. As a result of this the vigour of the trees has been greatly improved.

Bulmer's Norman, Yarlington Mill, Dabinett and Reine des Pommes, when grown as bush trees, continue to yield good crops under a variety of conditions. Bulmer's Norman, however, may soon prove to be unsuitable owing to the very vigorous growth that it is making at most of the centres. Michelin also crops well although, like Bulmer's Norman, it tends to be of moderate vintage quality. From the figures available it would appear that Ellis Bitter may just be coming into full cropping, fourteen years after planting. Sweet Alford, a noteworthy variety, cropped quite well in 1948 but in general it has still not come up to expectations. This may be due to its susceptibility to magnesium deficiency.

It is still too early yet to say whether the better known vintage varieties such as Foxwhelp, Silver Cup and Knotted Kernel, which are capable of giving good yields as standards, are going to be satisfactory as bush trees. Kingston Black and Cap of Liberty will presumably always prove difficult in the high rainfall areas because of canker. It is noteworthy that both Silver Cup and Kingston Black gave good yields at Faversham in 1949.

B. LONG ASHTON TRIAL.

Swarbrick in an earlier Report (2) has described the layout of the Long Ashton plot. Originally it was intended that the permanent plant on this plot should consist of 216 trees of each of the four varieties, Sweet Alford, Stoke Red, Dabinett and Knotted Kernel. It has since been found that 33% of the trees planted as Knotted Kernel are in fact the variety Woodbine and records for this variety have been included.

The trees are planted at 20' $\times$ 20' and thus each of the three main varieties occupies an area of about 2 acres. In conformity with the county centres half the trees were worked on Seedling Crab stock and half on Malling Type I, but one third of the trees were established with a 4' 6" leg and the remainder with one 2' 6" in length.

In December 1944 filler trees on rootstock Malling Type IX, interplanted black currants and trees of the variety Kingston Black were removed. Since 1946 cover crops and manurial experiments have been carried out on the plot and two reports on the progress of these experiments have been published by Bould in 1947 (4) and by Bould, Tolhurst and Jarrett in 1949 (5).

Yields 1945-9.

Table II gives the total yields for the years 1945-9 for the five varieties represented. These figures give a general indication of cropping capacity. However, in the compilation of these results no account has been taken of the differing cultural treatments which the trees have received. Because of these differences in treatment caution should be exercised when comparing these results with those obtained from other plots.

TABLE II.
AVERAGE YIELD—TONS PER ACRE (CALCULATED).
LONG ASHTON.

Year	Seasons after Planting	Sweet Alford	Stoke Red	Dabinett	Knotted Kernel	Woodbine
1945	10	0.1	0.84	0.96	Nil	Nil
1946	11	1.85	5.6	4.6	0.5	0.35
1947	12	2.05	0.55	1.4	0.55	0.04
1948	13	4.5	4.8	0.7	0.9	1.2
1949	14	8.3	3.2	6.2	1.1	1.05

Discussion.

Swarbrick (2) has already drawn attention to the good crops carried by Dabinett, and to a lesser extent by Sweet Alford, in the years 1941 and 1942. Comparison of the yields obtained from Dabinett on the Hereford, Faversham (Kent) and Long Ashton plots over the years 1947-9 show that this variety is consistently carrying the heaviest crops at Hereford. At this latter plot the trees have been planted 17.5' $\times$ 15' and, for a variety of medium vigour such as Dabinett, this closer planting favours a heavier yield per acre. At Long Ashton Dabinett has shown symptoms of potash deficiency and in the more severe instances this has affected tree growth and cropping. Sweet Alford continues to show symptoms of magnesium deficiency, but, in spite of the depression in yield which such a deficiency would be expected to produce,

there has been a great improvement in crop weight in each successive year since 1947.

The yield figures for Stoke Red tend to confirm the reputation of this variety for biennial bearing. Both Knotted Kernel and Woodbine are proving slow in coming into bearing. This is a characteristic feature of Knotted Kernel when it is grown as a standard tree.

SUMMARY.

An account is given of the cropping of certain cider varieties grown as bush trees at trial centres in the West of England and in Kent. Yields obtained on the Long Ashton Research Station trial plot are considered separately, because of the specialised treatments which this plot has received.

A. Trials at County Centres.

Details of cropping are given for two centres, one in Herefordshire and one in Kent, for the years 1947-9 and short notes on yields at the other centres in the West of England for the years 1948-9. Variations in yields due to season and to local conditions are briefly considered. Earlier conclusions that the varieties Bulmer's Norman, Yarlington Mill, Reine des Pommes and Dabinett consistently produce good crops are confirmed.

B. Long Ashton Trial.

Reference is made to the layout and history of the plot at Long Ashton. Total crop yields, irrespective of varying treatments, are given for the years 1945-9 for each of the five varieties, Sweet Alford, Stoke Red, Dabinett, Knotted Kernel and Woodbine. Certain cropping variations and characteristics as revealed by this trial are discussed.

ACKNOWLEDGMENTS.

We are grateful to Professor B. T. P. Barker for his helpful advice on the presentation of the data for this paper. We are also indebted to the following for supplying crop weight data:—

Mr. T. Neame, Faversham, Kent; Mr. E. Ball, Messrs. H. P. Bulmer & Co., Hereford; Mr. Stafford Weston, Much Marcle, Herefordshire, and Mr. R. M. Jarrett of Long Ashton Research Station.

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PROGRESS REPORT ON FRUIT BREEDING, 1949.

By G. T. SPINKS.

During the war years the production of new seedlings was suspended : it was also necessary to grub some of the old seedling trees which were still under trial and to raise young trees of these varieties. Consequently there has been a gap of several years during which little of fresh interest has been found in the breeding work though observation of previously selected seedlings has been continued. During the last few years, however, several families of seedlings have been raised and the young trees propagated from older selected trees have started to crop. Brief particulars of the work with different kinds of fruit are given in the following sections of this report.

Apples.

About thirty years ago a small family was raised from a cross between Allington Pippin and Star of Devon. There was no particular object in view when making this cross, which was one of a number made rather at random because suitable pot trees were available. In this family, however, two seedlings of considerable merit appeared and have now had a long period of testing. It was recently decided to name and distribute one of these and a short description of the new variety is given in the following paragraph.

Cheddar Cross. Dessert, late August to early September ; medium size, oval or slightly conical ; bright scarlet flush on clear yellow ground ; flesh yellowish, slightly crisp and moderately juicy ; fairly good flavour ; tree moderate in size and habit ; rarely affected by scab. This variety may be valuable owing to its very attractive appearance and its season, which is definitely before that of Worcester Pearmain. It is a good cropper but has a tendency to biennial bearing.

The other seedling from the same family as Cheddar Cross is receiving a further trial, as some judges prefer it to that variety, but it is not intended to name it at present : it is now known by its code number, A.S.D. 3. The fruit is round in shape and not so brightly coloured ; the quality is about the same and the season slightly earlier ; cropping has been good and rather more regular.

Three more seedlings (W.BB.35, W.BB.53 and W.BB.54) derived from a cross between Worcester Pearmain and Beauty of Bath appear distinctly promising as varieties to precede Worcester, but they have not yet had a lengthy period of trial and their cropping capacities are not known. All these sorts are of medium size, flattened round in shape and highly coloured. The fruit is about a fortnight later than Beauty of Bath, but larger, better coloured and of better quality.

Two October apples have attracted attention in the last few years. One, M.C.10 (American Mother $\times$ Cox), bears medium-sized fruit, round and regular in shape, with a rather dull scarlet flush ; the flesh is crisp and juicy and the flavour good. The fruit is excellent but it is rather doubtful whether the trees will crop heavily enough, as the branches show much bare wood : blossom is late. The fruit of the other variety, W.RL.28 (Worcester Pearmain $\times$ Rival), is of medium size, slightly angular, and highly coloured : quality is not as good as that of M.C.10 but is at least equal to that of Worcester. The

season probably extends from the beginning of October to the middle of November in an ordinary store. Cropping has been fairly good.

Several late dessert apples are still being tested but none of them are entirely satisfactory, being either poor croppers or bearing tough-fleshed fruit.

One cooking variety, A-104 (open-pollinated Allington) may be of value owing to its late-keeping qualities, but it is still doubtful whether the trees will crop heavily enough and whether the fruit is large enough.

During the last three years further batches of young seedlings have been raised with the object of producing good-quality dessert apples of really early and late seasons respectively. In an attempt to obtain an early sort the two varieties W.BB.35 and W.BB.53 (see above) were back-crossed with Beauty of Bath and two families, containing about 200 seedlings in all, have been raised. For a late variety the parents used were two selected seedlings, both of which blossom late and bear late-keeping fruit, but are not otherwise very satisfactory: they had been derived from (1) Cox $\times$ Sturmer and (2) Court Pendu Plat $\times$ Cox respectively. Only a small family has so far been raised from this cross.

Cider apples. There is a need for improved varieties, of different seasons, of some of the various classes of cider apples, such as the sweets, bitter-sweets and bitter-sharps. As yet, no attempt has been made to extend the range of sweet varieties but several families of seedlings have been raised in order to provide good new sorts of the other two classes. The seedlings raised are the product of crosses between Stoke Red (sharp) and the three varieties Frederick (sharp), Yarlington Mill (bitter-sweet) and Medaille d'Or (bitter-sweet), respectively. In all, about 500 seedlings have been produced from these crosses.

Pears.

The variety Bristol Cross continues to give satisfaction, both at Long Ashton and elsewhere. Though this variety has been said to be incompatible with quince stocks no cases of real incompatibility have yet been confirmed. It is true that a fairly large number of breakages at the union have occurred but these usually seem to take place when the young tree has just formed a good head and has started to crop. The union is apparently sometimes too weak to stand the strain at that time but it seems probable that efficient staking will enable the tree to survive the critical period and that there will then be no further risk of breakages at the union. A number of trees on quince stocks in our plantations are now twenty years old and are growing strongly and bearing heavy crops.

Cheltenham Cross is still satisfactory at Long Ashton but no reports on its performance elsewhere are yet available as it was distributed for the first time only three years ago. Another seedling variety of the same parentage—Dr. Jules Guyot $\times$ Conference—may prove to be as good as Cheltenham Cross but will not be named and distributed until it has had a longer trial: it is in season at the same time, mid-September, but differs in shape and colour.

Eighteen years ago three families consisting of 250 seedlings were raised with the object of obtaining late dessert varieties. The parents used were Comice, Bristol Cross and a late seedling derived from William's and Conference. Probably owing to poor growing conditions, cropping was much delayed and rather poor but finally 17 seedlings were selected for propagation. About half of these are late-keeping sorts: the remainder are earlier in season but have other good qualities: several bear fruit of notably large size. All the seedling trees have now been destroyed and as some of them were propagated

only at the time of grubbing it will be several years before confirmation of the qualities of the selected varieties can be obtained.

In continuation of the search for good late varieties two more families of seedlings have been raised in the last two years by crossing two of the above-mentioned late seedling varieties with Comice. The two families include about 180 seedlings.

Plums.

The late dessert varieties Severn Cross and Thames Cross, and the late cooker Frome Cross, continue to crop well at Long Ashton. Besides the named varieties Avon Cross, Teme Cross and Wye Cross, only four other seedling sorts now remain under trial, all of them being large dessert plums of late or fairly late season.

For a number of years no new families of plums have been produced and consequently there has not been a succession of new seedlings on trial. In 1948, however, the breeding programme included the production of improved early varieties and a start was made by crossing Early Laxton with a fairly early seedling of better quality but rather poor cropping capacity : the latter variety originated as a cross between Heron and McLaughlin's Gage. Up to the present time only a small family has been raised from this cross.

Blackcurrants.

Mendip Cross, Cotswold Cross and Malvern Cross are producing excellent crops each year at Long Ashton. All three varieties have been distributed and, Mendip Cross in particular, are being widely grown on a commercial scale. As Malvern Cross was distributed for the first time in 1946 few reports about it have yet been received.

For the last two years another sort, which appears to be rather later in season than Malvern Cross and a heavy cropper, has attracted attention. This was selected from a family produced as the second generation from Boskoop selfed : it has now been propagated for trial on a larger scale. Several more varieties are still under trial but it is doubtful whether they are equal or superior to the sorts already named.

In 1947, with the object of producing a currant late in season and containing a high content of Vitamin C, crosses were made between (1) Baldwin and Malvern Cross and (2) Baldwin and B.V. 140 (a late seedling variety derived from Baldwin $\times$ Victoria). Casualties among the seedlings were numerous owing to weather conditions and the families are not as large as could be wished : the surviving plants will fruit in 1950. Observations on growth and cropping will be made and the fruit of any plants apparently satisfactory in these respects will be analysed to determine the Vitamin C content.

General.

All the named varieties of the various fruits, and certain selected but un-named sorts mentioned above, have been included in either the main or preliminary trials of the National Fruit Trials.

Named varieties are available to growers and nurserymen who take advantage of the New Variety Distribution Scheme of the Station. Every variety has been distributed to some extent, but the most popular are the pears Bristol Cross and Cheltenham Cross, the plums Severn Cross and Thames Cross and the three blackcurrants. Supplies of some of these can now be obtained from nurserymen.

TREE SHAPE AND PRUNING TREATMENT IN RELATION TO CROP YIELD IN PEARS. PROGRESS REPORT.

By E. W. HOBBS and E. CATLOW.

A report (1) on pear growing at Long Ashton appeared in the Research Station Annual Report for 1944 and dealt with the performance of certain varieties of pear on various rootstocks. The present report concerns the records obtained from three plots laid down in 1937 and 1939 to compare methods of tree shaping and winter pruning and their influence on crop yields. The figures for yield etc. have not been subjected to statistical analysis, but the records show sufficient differences between treatments to make this report of practical value.

EXPERIMENTAL.

Pruning Treatments.

The winter pruning treatments involved have included :

- (a) Open centre shape with "regulated" laterals and leader tipping.
- (b) " " " " "spurred" " " "
- (c) Delayed open centre with "regulated" laterals and leader tipping :
- (d) " " " " "spurred" " " "

The several winter pruning treatments and the three varieties were disposed over three separate but closely situate plots as follows :

- Plot 1. Treatments (a) and (b) — varieties Conference and Laxton's Superb.
- Plot 2. Treatments (b) and (d) — variety Laxton's Superb only.
- Plot 3. Treatments (a), (b) and (c) — varieties Comice, Conference and Laxton's Superb.

Description of Terms.

Several of the terms used to describe the pruning adopted need explanation. Trees receiving regulated lateral treatment (Plate 1, Fig. 3) have been pruned very lightly. Each season a high proportion of lateral shoots have been left untouched and some of these have been reduced or cut out in later years after cropping. Overcrowding laterals have been cut out at the base. Leader tipping has been done according to shoot vigour and competing lateral shoots arising close behind the leaders have been cut out.

On the spurred trees lateral shoots over 3" long have been spurred back to about this length. Short laterals less than 3" long, often having a flower bud at the top, have been left unpruned. No spur thinning has been carried out to date.

The open centre shape trees (Plate 1, Figs. 1 and 4) need no special explanation. The maiden tree was headed originally at 2' 6" from the ground and a series of branches have been built up around a more or less open centre.

The delayed open centre trees (Plate 1, Fig. 2) have been built up on the lines described by Swarbrick (2) in his work on apple tree shape. A central shoot has been retained each year and has been pruned so as to keep the centre stem as upright as possible. This central leader has been shortened more or less severely according to tree vigour, and lateral shoots from the leader have

been selected each year and trained outwards to form the main limbs. To obtain shoots as conveniently and evenly spread as possible around the centre stem notching above suitable buds has been done at the time of the winter pruning, or unwanted buds have been cut out. The central stem was allowed to progress until the 8th or 9th year when the top of the tree was opened out in similar manner to that practised on the open centre shape tree. This "opening out" of the tree head was done at about 8' from ground level.

Leader tipping has been practised on all trees included in the trial. On the open centre trees tipping has been done at irregular heights around the trees. The amount removed each year had been just sufficient to produce a good breaking of lateral shoots and a fair degree of stiffening in the leaders. Hard leader tipping to produce a stiff framework has not been done.

On the delayed open centre trees leader tipping has aimed at producing a new leader growing outwards at a wide angle from the central stem. Tipping has usually been done to one or two buds *above* the desired bud pointing outwards; these one or two upper buds have then been notched below to check their growth, so encouraging the lower outward pointing bud to grow at a wide angle from the leader shoot. This technique helps to keep the branches depressed at a wide angle from the main stem.

The framework of delayed open centre trees has required more care in pruning than has the open centre bush tree and the amount of cutting necessary on the delayed open centre trees has been relatively greater than on the open-centre trees.

Tree Material.

In all, 172 trees are included in the records. All are growing on a fine sandy drift material a foot or so in depth derived from Keuper Marl. Drainage in all plots has been improved by tile drains.

All the trees are planted at 15' $\times$ 12' and were intercropped with black currants in the early years. Plot 3 was cleared of the intercrop in 1941 and Plots 1 and 2 in 1945.

Varieties and Rootstocks.

Three varieties are included, viz., Doyenne du Comice, Conference and Laxton's Superb, growing on rootstocks Quince A and Quince C (in equal number).

Cultivations.

During the early years soil cultivations were designed primarily for the benefit of the black-currant intercrop on all plots. Frequent spring and summer cultivations to suppress weed were followed in winter by shallow "ploughing up" of the tree and black-currant rows. After the removal of the black-currants the plots were seeded down, and the sward cut frequently by gang-mower.

Manuring.

Manuring consisted of annual dressings of inorganic N.P.K., at varying rates due to war-time conditions and to the changes in intercropping and grassing down.

Pest and Disease Control.

Annual winter washing consisted of a tar-oil wash at 5% up to 1942, since when a D.N.C. petroleum at 5% has been used.

The programme against scab has included 2 pre- and 2 post-blossom applications of lime-sulphur at 2% and 1% respectively, except on Doyenne du Comice which has had pre- and post-blossom lime-sulphur at 1%.

Records.

Tree development. Measurements of height, girth and spread of each tree included in the records have been taken annually. From these records the mean girth and spread of trees of each variety under each of the winter pruning treatments, after harvesting the eighth crop, have been calculated and these are shown in Tables I and II respectively.

Crop Weights. The crop of each tree in the records has been weighed annually over the past eight seasons (1942-1949 inclusive), the frost year of 1945 being included as a crop year. From these records the total crop has been calculated in bushels per acre for trees standing at 15' $\times$ 12' (i.e. 242 per acre) for each variety under each pruning treatment.

These data are shown in Table III.

RESULTS.

Tree shape and size in relation to pruning treatment.

The figures for mean girth and spread per tree in Tables I and II are set out under the two main treatments of Delayed Open Centre shape and Open Centre shape trees. Tree girth is generally accepted as an accurate indicator of tree development and in this case girth refers to the measurement of the main stem taken 12" above soil level. Figures for tree size in relation to pruning treatment of laterals are given under each of the two tree shapes.

The three varieties growing on Quince A and C, with open centre shape have received both regulated and spurred treatment of laterals. Trees of Comice and of Conference on Quince A receiving regulated treatment have a greater girth than trees receiving spurred treatment.

Conference on Quince C with regulated lateral treatment is also greater in girth than when laterals are spurred.

Laxton's Superb open centre, however, shows fairly similar girth measurement whether laterals are spurred or regulated.

Unfortunately, delayed open centre trees with spurred laterals and regulated laterals are not growing on the same plot but in this case girth measurement is in favour of the spurred tree.

The effect of pruning treatment on spread is marked in the case of open centre shaped trees. In every case the mean spread is greater where regulated lateral pruning is employed than where laterals are spurred, and this greater spread is related to cropping, as will be seen later.

On the delayed open centre tree, however, the more rigid formation of the limbs round a centre stem does not react to regulated lateral treatment in the same way, the spread being a little greater on trees where laterals have been spurred.

Crops from Delayed Open Centre and Open Centre Shape.

The crop yields from open centre trees, with the exception of Doyenne du Comice on Quince C, have been greater than those from delayed open centre trees. In the case of Conference on Quince A the crop from open centre trees was almost four times as great as that from trees of comparable age and environment, pruned as delayed open centre trees. Laxton's Superb on Quince A, open-centre yielded over twice the weight of crop obtained from

TABLE I.
MEAN TREE GIRTHS (INCHES) AT 12" ABOVE SOIL LEVEL. 1949.

Variety			Comice		Conference		Superb	
Rootstock			A	C	A	C	A	C
Plot 1.	Shape	Pruning						
	OC	R	—	—	12.4	10.9	12.5	12.2
	OC	S	—	—	11.0	9.3	12.6	12.1
Plot 2.	OC	S	—	—	—	—	14.0	12.4
	DOC	S	—	—	—	—	14.0	12.6
Plot 3.	OC	R	13.6	11.1	13.1	12.6	14.3	14.3
	OC	S	10.9	11.4	10.9	10.2	14.3	12.4
	DOC	R	11.5	11.2	10.4	8.7	11.9	10.4

TABLE II.
MEAN TREE SPREADS (FEET). 1949.

Variety			Comice		Conference		Superb	
Rootstock			A	C	A	C	A	C
Plot 1.	Shape	Pruning						
	OC	R	—	—	11.50	9.75	7.25	6.75
	OC	S	—	—	9.50	8.00	7.00	6.50
Plot 2.	OC	S	—	—	—	—	9.25	7.00
	DOC	S	—	—	—	—	9.00	7.25
Plot 3.	OC	R	7.25	6.75	13.00	10.00	10.25	9.00
	OC	S	6.50	6.50	9.25	9.75	9.00	7.75
	DOC	R	6.75	6.75	8.00	7.25	7.50	7.00

TABLE III.
MEAN TREE CROP WEIGHTS (BUSHELS/ACRE). 1942-49 INCL.

Variety			Comice		Conference		Superb	
Rootstock			A	C	A	C	A	C
Plot 1.	Shape	Pruning						
	OC	R	—	—	978	942	864	972
	OC	S	—	—	624	750	750	732
Plot 2.	OC	S	—	—	—	—	924	732
	DOC	S	—	—	—	—	618	618
Plot 3.	OC	R	144	180	1062	1032	1248	1164
	OC	S	126	162	792	876	942	906
	DOC	R	48	198	276	420	600	588

OC=Open centre.
DOC=Delayed open centre.

R=Regulated.
S=Spurred.

trees pruned to delayed open centre shape. Doyenne du Comice on Quince A open centre trees cropped just three times as heavily as comparable trees pruned to delayed open centre shape.

The comparisons for Conference and Superb on Quince C are almost equally striking.

Comice on Quince C cropped slightly more heavily on trees with delayed open centres than on trees of open centre form, but with this one exception no trees on either rootstock pruned as delayed open centre trees cropped as heavily as those trained in open centre form.

DISCUSSION.

The delayed open centre form of tree suggested itself at the outset of the pruning trial as being a useful shape to support a heavy fruiting framework. A centre stem might also provide a useful support to which branches could be strung up in the cropping season. None of the trees under review, however, whether of open centre or delayed open centre form, have been strung, so that their shape at the present time is directly due to the influence of the pruning treatment and the effect of crops weighing down the branches.

In the case of regulated trees branch breakage has been fairly common ; spurred trees have occasionally lost a branch but in no instance have branches been lost from the delayed open centre trees due to breakage by crop weight. All trees receiving regulated treatment have an untidy appearance and limbs tend to become twisted and out of place due to their relative whippiness compared with spurred trees.

In building the delayed open centre trees it has been difficult to produce an evenly balanced tree. The tendency has been for the branches on the windward side (West) to be bent upwards and so to present a flattened shape on the exposed side of the trees. The more vigorous the growth the greater has been this wind effect. Conversely lateral branch formation on the lee side has been relatively easy and trees have developed a satisfactory form on this side.

Where spurring of laterals has been practised on delayed open centre trees the effect of getting too upright a leader growth has been enhanced. Conversely, delayed open centre trees with regulated laterals have been built up much more satisfactorily. The leaving intact of lateral shoots along the limbs has kept the leaders growing outwards and the branches consequently developed at a wide angle to the main stem, resulting in a better proportioned tree although of lesser spread than where spurring was employed.

The three varieties under review have behaved very differently in the forming of flower buds.

Doyenne du Comice has spurred freely under any treatment. Flower buds have formed freely, generally speaking along extension growth and also on shortened laterals or on naturally formed spurs. Spurs have tended to die out, however, on the lower parts of limbs as they age, much more quickly than on either Superb or Conference. The habit of the variety is fairly upright, partly due to the effect of poor cropping, although spreading is noticeable on the regulated trees.

Conference has formed flower buds freely over the whole tree but more especially in bunches towards the tips of wood in its second year. In the spurred trees blossom buds have kept relatively close to the branches but in the regulated trees these bunches of flower buds tend to form well clear of the branches, giving rise to long whippy laterals which bend and swing about

PLATE I.



Fig. 1. Open centre; spurred.
Superb on Quince A.



Fig. 2. Delayed open centre; spurred.
Superb on Quince A.
Note flattened appearance of windward side of tree.



Fig. 3. Open centre; regulated.
Conference on Quince A.



Fig. 4. Open centre; spurred.
Conference on Quince A.

PLATE II.



The "Mapic" sub-soil injector.

when in crop. After the third year, extensions such as these become bare of blossom buds towards the bases and at this stage they need cutting out.

Laxton's Superb forms natural spurs very freely and these increase in size in succeeding years, becoming very complicated. The spurs are fine-wooded, brittle and break easily, and during pruning it is essential therefore to thin out such spurs occasionally in order to reduce numbers of flower buds and weight of fruit on the branches. During the trial spurs have been allowed to increase in size unpruned but drastic spur thinning is now due. Superb is a marked biennial bearer and without spur thinning this tendency is likely to be encouraged. Unless some young laterals are left in each year the trees become furnished with an ever aging spur system and this also may increase the tendency to biennial bearing.

Fruit crops to date have been much heavier from trees of open centre shape but now the formative years of the delayed open centre trees are, generally speaking, completed, the cropping difference may even out.

No account of fruit quality has been taken in the records but it is intended to make records of this in future.

SUMMARY.

A trial is described of pears, trained as open centre bush trees and as delayed open centre trees, which have received spurred or regulated treatment of laterals.

Delayed open centre trees, whether spurred or regulated, have produced smaller crops than have open centre bush trees of the varieties Superb and Conference.

Tree size has been influenced by the shape, open centre trees being greater in overall size than delayed open centre trees.

Regulation of laterals on open centre trees has resulted in a marked increase in cropping: on delayed open centre trees the position is reversed but not to the same extent.

On delayed open centre trees a better formation of limbs has been obtained by regulated treatment of laterals than by spur pruning.

ACKNOWLEDGMENTS.

The writers are indebted to Mrs. R. Lewis and Miss O. M. Woodhouse who have carried out much of the recording and pruning of the trees under trial.

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NUTRIENT PLACEMENT IN RELATION TO FRUIT TREE NUTRITION.

II. EXPERIMENTS ON SUB-SOIL INJECTION.

By J. TOLHURST and C. BOULD.

In 1948 preliminary experiments were begun to study the technique of sub-soil injection of fertiliser solutions, as one of the methods of applying mineral nutrients to fruit trees (1). Two lines of study were followed, one dealing with the distribution of the fertiliser in the soil, the other with the effect on the nutrient status of the tree.

A. Injection Apparatus.

The "Mapic" model used is manufactured in France,* and is intended for use with a spray line, "a" (Plate II). A hand-operated two-way port, "b," feeds the solution to a 250 c.c. cylinder, "c," containing a two-way piston. Thus at each movement of "b," 250 c.c.s. of solution are forced down the hollow pointed lance, "e", and out of four one-eighth inch holes near the tip. The lance may be forced into the soil to a depth of sixteen inches by pressure on the foot-rests, "d."

B. Soil Studies.

1. *Effect of volume and soil type on the movement of potassium in the soil.*

Sub-soil injections of a 10% solution of potassium chloride, at 12" below the surface and at 75 lbs. per sq. inch pressure, were made at three centres. At each, injections were made with two and four litres per hole in triplicate. The three soil types were:—

- (a) A stiff clay, recently cultivated, with poor drainage and intense gleying.
- (b) A clay loam, under permanent grass, with impeded drainage.
- (c) A coarse sandy medium loam, under grass for three years, with free drainage.

The moisture content at the time of injection at centres (b) and (c) was 80% saturation, whereas at centre (a) saturation was 88% of the maximum water holding capacity. Samples were taken with a tube auger at 12" depth at horizontal intervals of 4, 8 and 12 inches from the injection hole. As the injector lance has two pairs of holes, with axes at right angles to each other, sampling was done along each of the axes.

Determination of readily available potassium, using Morgan's reagent as extractant, two days after injection showed no consistent differences in penetration of potassium between the two and four litre treatments, nor between soils (b) and (c). In these two soils, at 12" depth, there was a high concentration of potassium 4" away from the point of injection, but very little at 8" distance. With soil (a), where the gleying provides large channels, the potassium at 4" distance was similar to (b) and (c), but at 8" there was still a medium concentration. There was no difference in distribution along the two axes.

* 100, Boulevard Pereire, Paris, 17e

2. *Effect of pressure on distribution.*

Injections of 20% potassium chloride at 15" depth and at 200 lbs. per sq. inch were made in triplicate with volumes of two and four litres per hole. After twenty-four hours, sampling was done from a rectangular pit, dug so that a cane marking the injection hole was at one corner and two faces of the pit were in the vertical planes of the axes of injection. Small cores were taken at 2" intervals from the cane in a horizontal direction at depths of 6, 9, 12, 15, 18 and 21 inches. The samples were prevented from drying out, and were analysed for available potassium. The results showed that 10" was the greatest horizontal penetration, this being at 15" depth and on the injection axes. At 6" depth, and also at 21", penetration was only 6" horizontally. At intermediate levels the spread of potassium was between 6 and 10 inches.

The soil was a fine sandy medium loam, compact, and with a moisture content of 88% of the maximum water holding capacity.

3. *Effect of volume, pressure and soil type on surface seepage.*

In a friable loam 3 litres could be injected at 400 lbs. per sq. inch before liquid escaped at the surface, and 5 litres at 200 lbs. per sq. inch.

In a compact loam with a pan of 12" depth, surface seepage occurred after injection of half a litre at 200 lbs. per sq. inch.

From other observations it would appear that the controlling factor is soil compaction at the point of injection, rather than moisture content or pressure of application.

C. Fruit Tree Studies.

All injections were made within the drip area of the branches, i.e. approximately on a circle with radius equal to branch spread.

1. *Effect of fertiliser concentration on tree roots.*

A solution containing ammonium sulphate, ammonium phosphate and potassium chloride was injected round three standard apple trees, 15-year old Monarch on Bulmer's Norman growing under grass. 4 litres per hole were applied with four-fold replication at concentrations of 5, 10 and 20% W/V.

After three weeks, and again at twelve weeks, pits were dug round the marked injection holes and observations made on the condition of the roots. No damage was observed at any concentration of fertiliser. After twelve weeks there appeared to be a congregation of roots round the point of injection. Each tree received 7 lbs. of fertiliser. The foliage showed no adverse effects due to treatment.

2. *Effect of injection on young stunted trees suffering from severe potassium and magnesium deficiency.*

These Lane's Prince Albert trees, under clean cultivation, had made no growth for some years, and in an attempt to remedy this, injections of various combinations of nitrogen, phosphorus, potassium and magnesium in 20% solution were made at four points round each tree on April 23rd, 1948.

No effect on leaf symptoms or shoot growth was visible even after two seasons.

3. *Effect of injection of magnesium sulphate on the uptake of magnesium by the tree.*

Twelve young Laxton's Superb bush apple trees on M.II growing under grass were treated with calcined Kieserite, $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, in 10% solution at

two rates, $2\frac{1}{2}$ and 5 gallons per tree, applied in 5 holes at 100 lbs. per sq. inch pressure. Injection was made on April 23rd, 1948. Leaf samples were taken at fortnightly intervals from the young growth, for magnesium determination by full chemical analysis. No differences between the controls and treated trees were observed, either from the 1948 analyses or from the one analysis in 1949. The magnesium status of the trees was low.

4. *Comparison of sub-soil injection with other methods of applying potassium to plum trees.*

Twenty Blue Tit plum trees, four years old, growing on Pershore heavy sand and all showing severe symptoms of potassium deficiency in 1948 were treated with potassium in 1949. The treatments were: control; surface application of 3 lbs. potassium chloride per tree; sub-soil injection of a 15% solution to give the same weight of potassium chloride; and three foliage sprays of 2% potassium sulphate. The treatments were arranged in five randomised blocks.

Leaves from non-fruiting spurs were sampled on August 5th, 1949 and analysed for potassium. The means for each treatment are given in Table I:—

TABLE I.
EFFECT OF METHOD OF APPLICATION ON UPTAKE OF POTASSIUM
BY PLUM, VAR. BLUE TIT.

Treatment	(1) Control	(2) Surface	(3) Sub-soil	(4) Spray	Mean	Sig. Diff. at P=0.05
Mean % K ₂ O in Dry Matter	0.927	1.716	1.872	1.547	1.516	0.732

It will be seen that while treatments (2) and (3) have increased the potassium uptake, treatment (4) just falls short of significance. Sub-soil injection is not significantly superior to surface application or to foliage spraying.

It should be noted that when the samples were taken, the marginal scorch on the leaves of the controls had become so severe that a fringe of dead tissue fell off, and the remaining portion of the leaves may have contained more potassium than an intact, scorched, leaf. This would minimise the differences between the controls and the three treatments.

Cultivations in this orchard moved the soil to a considerable depth after the two soil treatments had been applied, and probably brought the surface applied fertiliser into contact with the roots.

5. *Effect of sub-soil injection on lime-induced chlorosis of plums.*

Fourteen Purple Pershore plums about 20 years old and growing on clean cultivated Evesham clay showed severe chlorosis in 1948. On February 23rd, 1949, five were treated with solid ferrous sulphate injected as pills into the trunk, and four were treated by sub-soil injection with a pseudo-solution of sodium ferri-silicate, prepared according to Demolon and Bastisse (2).

Visual observation on May 13th, 1949 showed that only the trunk injections were having a beneficial effect, while on June 22nd three of the trees sub-soil injected, and all five of those trunk injected, were decidedly improved. By early August the chlorosis had disappeared from all the trees including the controls, a seasonal effect.

6. *Magnesium uptake by apples comparing various sub-soil injection patterns with surface applications.*

The trees used were 20-year old Lane's Prince Albert on mixed rootstocks, under weed mulch, which had not had fertiliser for a year and were low in magnesium. Nine treatments were employed, arranged in eight randomised blocks. The object was to find the best pattern of injection for rapid uptake of magnesium. Magnesium sulphate (Epsom Salts, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) was applied to each tree in the following treatments:—

(1) Control; (2) 5 lbs. Epsom Salts in 10% solution injected in 5 holes spaced evenly round the drip-circle; (3) the same quantity of solution but in 10 holes; (4) the same quantity of solution but in 15 holes; (5) the same quantity of solution in 10 holes arranged in two bands of 5 on either side of a tree; (6) the same quantity of solution in 15 holes arranged spirally from the trunk to just outside the drip-circle; (7) surface application of 5 lbs. of solid Epsom Salts, broadcast; (8) solid surface application of 5 lbs. Epsom Salts in a band round the drip-circle; (9) as in (8) but at half rate, i.e. $2\frac{1}{2}$ lbs.

Injection was done on April 3rd, 1949 at a pressure of 220 lbs. per sq. inch and at 15" depth. Dry weather had cracked the soil deeply and solution escaped up the cracks, more especially in treatments where large volumes were injected into each hole.

Leaves from the leader growth were sampled on April 24th, 1949 and August 29th, and analysed for magnesium. The first sampling showed a good uniformity. The means of the treatments for the second sampling are given in Table II.

TABLE II.

EFFECT OF METHOD OF APPLICATION OF MAGNESIUM SULPHATE ON THE UPTAKE OF MAGNESIUM BY APPLE, VAR. LANE'S PRINCE ALBERT.

MAGNESIUM IS EXPRESSED AS % MgO IN LEAF DRY MATTER.

Treat- ment	Control	5 holes circle	10 holes circle	15 holes circle	10 holes two bands	15 holes spiral	5lbs sol- id broad- cast	5lbs sol- id band	$2\frac{1}{2}$ lbs. solid band
MgO	0.288	0.309	0.298	0.318	0.328	0.305	0.349	0.292	0.303

Analysis of variance showed that differences between treatments were not significant.

DISCUSSION.

The experiments described above were designed to gain experience of the technique of sub-soil injection of fertiliser solutions, prior to laying down more comprehensive trials for comparison of injection with other methods of fertiliser placement.

While the distribution in the soil of injected solutions was more restricted than was anticipated, it is felt that this was not sufficiently so to account for the frequent lack of response of treated trees. The pressure of application affected the speed of working but not the spread of the injected solutions.

It had been hoped that the formation of several concentrated pockets of fertiliser round a tree, in an area where root activity is known to be high, would result in a rapid uptake of nutrients. This would have been of particular value where cultural or soil conditions retard or even inhibit the penetration of

broadcast fertilisers to the region of fruit tree roots. The trials done so far have not shown an enhanced uptake from sub-soil injection.

In view of the exploratory nature of the work described it is not possible to give conclusive judgment on the value of sub-soil injection as a means of applying nutrients to fruit trees.

SUMMARY.

1. The "Mapic" sub-soil injector is described and illustrated.
2. Results are given for the distribution of potassium chloride solution in the soil with variations in volume and pressure of injected liquid.
3. Factors affecting surface seepage of injected solutions are discussed.
4. The effect of varying concentrations of fertiliser solution on tree roots is described.
5. The effect of sub-soil injection on growth of very stunted trees is described.
6. Results are given for the uptake of magnesium by apple, var. Laxton's Superb.
7. A comparison with other methods of application of potassium to plum, var. Blue Tit, is tabulated and discussed.
8. The effect of sub-soil injection on lime-induced chlorosis of plum, var. Purple Pershore, is described.
9. Results are tabulated for a randomised experiment to compare various methods of application of magnesium to apple, var. Lane's Prince Albert.

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ZINC AND COPPER DEFICIENCY OF FRUIT TREES.

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In July, 1948 attention was directed to a serious failure of apple trees on a plot used in the National Fruit Trials, at the Royal Horticultural Society's Gardens, Wisley. Many trees were showing symptoms of magnesium deficiency, some also manganese deficiency and severe rosetting of leaves and tip die-back of shoots. As a result of investigations we have been able to show that the rosetting is due to zinc deficiency and the summer tip die-back to a deficiency of copper.

Preliminary reports of these investigations have been published elsewhere (2) (3). Although the experiments on apples are still in progress, and others have been started with pears, it is felt that the symptoms of zinc and copper deficiency, together with tentative treatments for their control, should be brought to the notice of advisory officers and growers.

At the time these results were obtained no serious deficiency of zinc or copper in fruit trees had hitherto been shown to occur in Great Britain.

SITE AND SOIL.

The site is low-lying and level, and the soil is of light sandy texture overlying coarse sand and gravel. The pH value (following liming treatment to correct acidity in 1943-44) in the surface soil varies from 6.5 to 7.5 and decreases with depth. Citric soluble phosphate (P_2O_5) is high, ranging from 0.06% to 0.09% in the 0-6" layer and falling to half these values between 6-12". Organic matter is low, varying from 1.8% in the 0-6" layer to 0.5% in the 6-12" layer. The results of chemical and biological tests (*Aspergillus niger*) for zinc and copper on soil samples are given in Tables I and II. The soil samples for the determination of the two metals were taken from adjacent sites in the same field.

TABLE I.
ZINC CONTENT OF AFFECTED (WISELEY)
AND NORMAL SOIL (LONG ASHTON).

Soil	Zn as μ g per g. of air-dry soil			
	<i>Aspergillus niger</i> test	Soluble in N Ammonium acetate at pH 7.0	Soluble in N Ammonium acetate at pH 4.5	Soluble in cold 0.1 N Hydrochloric acid
Wisley soil 0-6"	1 to 2	1.3	8.9	10.0
Long Ashton soil 0-6"	10.0			

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TABLE II.
COPPER CONTENT OF AFFECTED (WISELEY)
AND NORMAL SOIL (LONG ASHTON).

Soil	Cu as μg per g. air-dry soil	
	<i>Aspergillus niger</i> method	Soluble in cold 0.1 N HCl
<i>Wisley soils</i>		
Gloucester Cross Area 0-6"	2.0	1.6
Crane's Seedling Area 0-6"	0.6	1.0
Crane's Seedling Area 6-12"	0.2	0.9
<i>Long Ashton soil</i> (For comparison)	25.0	

ZINC DEFICIENCY.

Symptoms.

Apples. The shoots on affected apple trees carry sparse foliage and show typical short internodal growths with leaf rosetting and shoot die-back (which usually occurs in the winter); the leaves are much reduced in size (so called little leaf condition), and tend to be lanceolate, with wavy margins and diffuse intervenal chlorosis, and often have a narrow dark-green marginal rim (Plate III). The waviness and upward curling of the leaves is more pronounced on broad-leaved varieties of apple. Shoots and spurs are markedly brittle and the bark of older growths somewhat rough. Affected and normal branches often occur on the same tree, and rosetted spurs may produce normal growth later in the season. The die-back occurs during the winter on rosetted branches. This is a point of difference from summer die-back due to copper deficiency which affects the current year's terminal shoots.

Several varieties of apples occur in the worst affected area, among which two varieties, Laxton's Pearmain and Edith Hopwood, were selected for experimental treatment. Unlike copper deficiency, zinc deficiency does not appear to be especially associated with any particular rootstock.

Pears. The symptoms of zinc deficiency in pears are less pronounced than those in apples. The leaves are much reduced in size, shoots become rosetted and the leaves show a diffuse intervenal chlorosis and a tendency to upward curling. A striking feature in pears is the large proportion of dead bark tissue on the main stems and branches. This symptom, however, may be accentuated by copper deficiency; the pears at Wisley are known to be also very low in copper.

Zinc Content of Leaves and Shoots.

Leaves and shoots from healthy and affected branches were taken for chemical analysis. The results given in Table III indicate that when the zinc content of apple leaves and bark is less than 3 p.p.m. in dry matter deficiency symptoms and rosetting occur. Leaves and bark from healthy trees in the affected area contain about 10 p.p.m. of zinc. This value, although not producing leaf symptoms, may not be optimum for apples. Thomas *et al.* (6) state that the zinc content of apple leaves below which deficiency symptoms appear

is less than 14 p.p.m. in dry matter. They found that the zinc content of apple leaves in August varied from year to year in the same trees. In 1945 the zinc content of healthy leaves ranged from 14 to 52 p.p.m. dry matter and in 1946 from 30 to 65 p.p.m. dry matter.

TABLE III.
ZINC CONTENT OF LEAVES AND SHOOTS FROM
HEALTHY AND AFFECTED APPLE TREES. 28/7/48.

Variety	Plant portion tested	Zn as $\mu\text{g.}$ per g. or p.p.m. in dry matter
Laxton's Pearmain (healthy)	Leaf	9.6
Laxton's Pearmain (little leaf)	"	1.2
Edith Hopwood (little leaf)	"	2.3
Lord Hindlip (healthy)	Bark	10.8
Lord Hindlip (little leaf)	"	2.1

Treatment. Tentative Methods.

Trunk or branch injection of solid zinc sulphate, and dormant and foliage sprays of this substance, are all effective in correcting zinc deficiency when carried out at the appropriate time and using specified amounts and concentrations. When the salt is injected in the solid form during the dormant season at $1/5$ and $1/2$ the rate recommended by Bennett (1) for iron sulphate in the treatment of iron deficiency in cases of lime-induced chlorosis, it is effective in controlling deficiency symptoms and causes no damage.

A dormant spray of 4% zinc sulphate applied in February will control symptoms in the same year, but a 2% dormant spray is only partially successful. No damage is caused by these dormant sprays.

Dilute solutions of zinc sulphate (0.1 to 0.2% concentration) when used with an effective wetting agent at petal fall, or shortly afterwards, are effective in correcting deficiency symptoms and one spray is sufficient for the season when applied at this time. If the spray is applied later in the season, from July onwards, little or no response is obtained in that year but sufficient zinc is absorbed to produce normal growth in the following year. Foliage sprays at 0.2% concentration applied in June may cause slight to moderate leaf damage to certain apple varieties, and severe defoliation of certain pear varieties.

Sufficient evidence is not yet available on the residual effect of foliage sprays, or on the compatibility of zinc sulphate with routine sprays. These problems are being investigated.

COPPER DEFICIENCY.

Symptoms.

Apples. The first symptoms appear in early July on the terminal leaves of current year shoots in the form of large irregular necrotic areas, and these are followed by upward-curling and distortion of the leaves. The lower leaves on affected shoots are usually pale green and sometimes exhibit numerous small, irregular necrotic spots. One variety in addition showed an irregular intervenal chlorosis, but this symptom is not general. Towards the end of July the shoots begin to defoliate, from the tip downwards, and from September

onwards they begin to wither and die (Plate IV). The bark of the main stem is usually very rough and may contain large necrotic areas.

Trees on Malling No. XII rootstock appear to be much more susceptible to copper deficiency than trees on Nos. I and II. In this particular plantation most of the trees on No. XII rootstock had died.

Pears. The symptoms on pears are similar to those of apples. They first appear on the terminal leaves of young current season growths. The bark of pears deficient in copper becomes very rough, deeply fissured, and is followed by necrosis and shedding.

Copper Content of Deficient and Healthy Foliage.

The copper content of leaves from healthy and diseased apple trees is shown in Table IV.

Deficiency values for leaves from the middle region of terminal shoots appear to be below $5\mu\text{g}$. of Cu per gm. or as p.p.m. of dry matter. The figures agree with those of Oserkowsky (5) and Dunne (4).

Treatment. Tentative Methods.

Responses have been obtained to injection of solid copper sulphate at the same rate as for zinc sulphate and to foliage sprays of 4:4:50 Bordeaux mixture. Further experiments are in progress to test soil application of copper sulphate and foliage sprays of dilute copper sulphate and Bordeaux mixtures. Until these results are available no definite recommendation for the best treatment of copper deficiency can be given.

TABLE IV
COPPER CONTENT OF LEAVES FROM HEALTHY AND DEFICIENT
APPLE TREES. (SAMPLES TAKEN 30/8/49).

Variety	Cu as μg per g. of leaf dry matter	Comments
<i>Wisley</i>		
Crane's Seedling on Malling XII	2.0	Diseased
Gloucester Cross on Malling XII	2.2	Diseased
Gloucester Cross on Malling XII (Injected 1948)	5.1	Healthy
Cellini Pippin on Malling XII	2.5	Diseased
<i>Long Ashton</i>		
Allington Pippin	6.0	Healthy
Lane's Prince Albert	5.7	Healthy
Edward VII	5.7	Healthy

SUMMARY.

1. The symptoms of zinc and copper deficiency in apples and pears as recorded at Wisley are described and soil data for the two elements from healthy and deficiency areas are given.

PLATE III.



A.

B.

C.

Zinc deficiency in apple.

A. Normal shoot; var. Edith Hopwood.

B. Zinc deficient shoot; var. Edith Hopwood. Note rosetting, upward curling and waviness of leaf margins and interveinal chlorosis.

C. Rosetted shoot; var. Laxton's Pearmain.



A. First symptoms—appearance of necrotic areas on the terminal leaves of current year's shoots.
 B. Distortion of terminal leaves and onset of defoliation.
 C. Summer die-back of shoots (wither tip).

2. Leaves taken from rosettes on zinc deficient trees contain about 2 to 3 μg of Zn per gm. (or as p.p.m.) of dry matter as compared with 10 or more p.p.m. in healthy leaves.

3. Leaves taken from the middle region of terminal shoots on trees showing summer die-back (copper deficiency) contain less than 5 μg . of Cu per gm. of dry matter.

4. Tentative methods for the control of zinc and copper deficiencies in fruit trees, as indicated by results at Wisley, are given.

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EXPERIMENTS ON IRON METABOLISM IN PLANTS

II. THE INTERRELATIONSHIP OF IRON AND POTASSIUM IN THE METABOLISM OF THE POTATO PLANT.

By E. W. JONES and E. J. HEWITT.

The work described in this paper was carried out in 1947 and was based on preliminary observations made in 1945 and 1946, when it was observed that visual symptoms and yields of crops grown at any one iron level varied greatly according to the amount of potassium present in the nutrient. The reciprocal effect of the iron supply on potassium deficiency was also suspected although not, however, clearly defined on the basis of either yield data or tissue test results. In view of the importance of this interrelationship and its possible bearing in the problem of lime-induced chlorosis (6) (14), a more complete experiment, although essentially still exploratory in character, was laid down in 1947. The potato was chosen as a suitable experimental plant since (a) it exhibits easily recognisable and well defined symptoms of both iron and potassium deficiencies, (b) both elements were thought to be factors concerned in the blackening of tubers on boiling, and (c) it is of economic importance.

The more recent literature conveys little specific information on the interrelationship of iron and potassium in plant metabolism and many of the references cited in the bibliography contain incidental remarks only, but provide sufficient evidence to suggest that the problems of iron nutrition are frequently related to the potassium status of the plant (1, 2, 4, 7, 10, 12, 13, 15, 17, 18).

EXPERIMENTAL

Three levels of iron were given at each of three levels of potassium. This gave a total of nine treatments; each comprised ten pots. These levels were

chosen to represent deficiency, normal and luxury consumption or excess. These were (in terms of milliequivalents per litre):— $\text{Fe}_1 = 0.0003$; $\text{Fe}_2 = 0.30$; $\text{Fe}_3 = 1.50$; $\text{K}_1 = 0.08$; $\text{K}_2 = 0.8$; $\text{K}_3 = 8.0$. The remaining nutrient elements were added in the following concentrations (expressed as milliequivalents per litre): $\text{Ca}^{++} 8.0$; $\text{Na}^+ 6.33$ (K_1 and K_2), 1.33 (K_3); $\text{Mg}^{++} 3.0$; $\text{PO}_4''' 4.0$; $\text{NO}_3' 13$; $\text{SO}_4'' 3.0$ (K_1), 3.72 (K_2), 5.92 (K_3); $\text{Mn}^{++} 0.02$; $\text{Cu}^{++} 0.002$; $\text{Zn}^{++} 0.002$; $\text{BO}_3''' 0.01$. Ammonium molybdate was supplied at a level of 0.001 millimol per litre. The nutrients were freed from iron by the phosphate adsorption method (11). The general sand culture technique used in this experiment has been described elsewhere (3). The potato variety used was "Majestic" of certified Scotch seed stock; the tubers were sprouted normally and planted about 3" deep during the last week of May.

The young and old leaves were sampled twice during the season. The young leaves were sampled at the third and fourth nodes from the shoot tip, while the "old" leaves were those taken from the mid-stem region; these were not senescent leaves but showed typical early potassium deficiency effects in the K_1 and K_2 levels or the "paling off" effect (cf. below) in the K_3 treatment. Thus foliage was sampled in regions which were visually most responsive to the different levels of iron and potassium supply. The potatoes were harvested on the 15th August. The haulms were cut at sand level and weighed fresh; the tubers were washed, dried and also weighed fresh. The soluble nutrients were determined by tissue test methods already published (8).

RESULTS

Visual Observations.

One month after planting typical iron-deficiency chlorosis was observed in the Fe_1K_1 and Fe_1K_2 treatments. At first the chlorosis appeared in the usual manner—the veins remained green and the intervenal regions became chlorotic. Later, however, the chlorosis was markedly accentuated and the whole lamina was chlorotic; this chlorosis in the K_1 and K_2 treatments was localised quite distinctly in the young shoot region. At this time the Fe_1K_3 plants showed a more diffuse general paling without any sharply defined chlorosis. They were taller and more vigorous than at the K_1 and K_2 levels. The lower leaves were paler than the youngest leaves which were of a normal green colour. The severity and distribution of the chlorosis and the general vigour of the plants were evidently governed by the potassium status. (Plate V, Fig. 1).

The chlorosis in the Fe_1K_1 treatment was accompanied by severe potassium deficiency symptoms in the old leaves; a buff coloured necrosis also appeared on the upper side of the midrib of the terminal leaflets in the young leaves and in triangular areas between the major veins or adjacent to the midrib in the middle of the leaflets. This type of necrosis was scarcely obvious in the Fe_1K_2 treatment but mild potassium deficiency symptoms were present in the older leaves. There were no potassium deficiency symptoms in the Fe_1K_3 plants. The symptoms of potassium deficiency as they appeared in the midstem leaves were quite typical (and have been previously described (16)). Potassium deficiency symptoms were first seen in the Fe_1 treatments and were followed by the Fe_2 and lastly by the Fe_3 set. Increasing amounts of iron *delayed* these symptoms but when they had appeared their *ultimate* severity was greater with increased iron level. At all iron levels potassium deficiency symptoms were recorded in the K_1 and K_2 treatments only, and in

the high iron types scorching spread rapidly to the shoot tip. No necrosis was, however, seen on the veins of the upper leaves as described for the low iron plants. Plants grown at the Fe_2 level were a normal green colour; those at the Fe_3 level were dark green, especially when potassium was deficient.

Table I shows the height recorded at two different dates. The August set show that increased potassium supply increased the height. The effect of increased iron supply when potassium was limited, as in K_1 treatments, was to increase the height; in the K_3 treatments where potassium was not limited, however, a slight decrease was recorded.

Yield Data.

Yield data are given in Table I. (The term "total" includes aerial shoots and tubers only). Increase of potassium at any iron level caused a distinct increase in both total and tuber fresh weights, except at the Fe_1 level, where tubers weights of the K_2 treatment were less than those of the K_1 . Increased iron supply at any one potassium level increased the total yield. The increments were far greater from Fe_1 to Fe_2 than from Fe_2 to Fe_3 . Similar results were obtained for tuber weights, which were small due to the early harvesting.

TABLE I.

WEIGHTS (IN GMS) AND HEIGHTS (IN CMS.) OF POTATO PLANTS GROWN WITH DIFFERENT POTASSIUM AND IRON LEVELS.
(RESULTS FOR 10 POTS).

Treatment		K level								
Fe Level	Date	K_1			K_2			K_3		
		Mean Height	Total wt.	Tuber wt.	Mean Height	Total wt.	Tuber wt.	Mean Height	Total wt.	Tuber wt.
Fe_1	27/6/47	22.3			29.0			39.3		
	14/8/47	27.8			47.0			98.8		
	15/8/47		24	4.8		146	2.0		872	6.7
Fe_2	27/6/47	27.8			33.0			41.9		
	14/8/47	41.8			61.0			91.5		
	15/8/47		245	4.6		915	7.8		3361	18.3
Fe_3	27/6/47	30.0			36.8			40.8		
	14/8/47	44.8			55.8			90.3		
	15/8/47		371	9.3		1197	16.0		3923	30.0

Tissue Test Data.

The results of the tissue tests are shown in Table II. The hydrochloric acid extracted more iron than the Morgan's reagent. Generally the Morgan's extracts gave more consistent results and showed an increase in iron content with increase of either iron or potassium supply.

Increased potassium concentrations in the nutrient solution were paralleled (with one exception) by increased soluble potassium in the foliage (petioles). The total extractible potassium was reduced at the later sampling in plants grown at the K_1 level but little seasonal change, or slight increases, occurred when a high level was given. Increases of iron at the low potassium levels tended to reduce the extractible potassium but there was less effect at the highest level.

The soluble calcium values varied inversely with the level of potassium in the nutrient solutions and in the Morgan's extract. For the higher levels of iron and potassium, increase in the iron level decreased the extractible calcium.

The ratios for old to young leaves of soluble iron, potassium and calcium are given in Table III. At the first sampling the acid extracts of old leaves usually contained markedly more iron than the young leaves at the K_1 and K_2 levels. At the later sampling for the K_2 and K_3 levels there was no difference between young and old leaves; the Fe_2K_1 , Fe_3K_1 and Fe_1K_3 treatments were notable in that the old leaves contained less iron than the young. The Morgan's extracts of old leaves contained as much or more iron than the young.

The old leaves generally contained less soluble potassium than the young. But there was a marked increase in the ratio (old/young) with increased iron supply at the K_1 level, and smaller, but generally consistent, increases at the K_2 and K_3 levels. This ratio, with one exception ($Fe_2 K_2$), also increased with increased potassium supply.

The old leaves contained more calcium than the young; at the higher K levels the old/young calcium ratio tended to be high (Table III).

TABLE II.

TISSUE TEST DATA FOR SOLUBLE NUTRIENT ELEMENTS AS P.P.M. IN PETIOLE EXTRACTS.

Treat- ment		K Levels											
		K_1				K_2				K_3			
		HCl Extract	Morgan's Extract			HCl Extract	Morgan's Extract			HCl Extract	Morgan's Extract		
Date	Leaf Sam- pled												
Fe Levels		Fe	Fe	K	Ca	Fe	Fe	K	Ca	Fe	Fe	K	Ca
Fe_1	Old	0.8	0.3	10	400	1.0	0.3	60	NIL	1.0	0.3	200	200
	Young	0.8	0.2	200	275	0.6	0.2	250	275	0.4	0.3	300	10
Fe_2	Old	1.2	0.4	30	275	1.8	0.4	50	275	1.4	0.6	50	100
	Young	0.4	0.2	150	275	0.8	0.4	200	180	3.0	0.3	250	10
Fe_3	Old	1.4	0.4	100	300	2.0	0.6	40	300	2.0	0.6	250	50
	Young	0.8	0.3	30	180	2.4	0.4	50	—	3.0	0.8	250	10
Date													
5/8/47													
Fe_1	Old	4.0	1.0	15	250	4.0	1.2	25	225	4.0	1.6	250	200
	Young	2.0	0.2	150	150	4.0	0.3	200	200	0.8	0.4	400	NIL
Fe_2	Old	0.8	1.0	10	300	0.6	2.0	15	250	0.6	1.8	300	75
	Young	2.0	0.4	25	200	0.6	0.5	50	275	0.6	0.8	150	NIL
Fe_3	Old	0.8	2.0	10	250	0.6	1.6	20	200	0.6	2.0	300	NIL
	Young	2.0	0.5	10	150	0.6	0.5	75	75	0.6	1.0	250	NIL

Ash Analysis Results.

The results obtained for total analysis of dry matter of the leaves are presented in Table IV.

Increased supplies of potassium increased the potassium content. At

TABLE III.
RATIOS OF SOLUBLE NUTRIENTS IN OLD AND YOUNG LEAVES.

Treatment	K Level											
	K ₁				K ₂				K ₃			
	Fe		K	Ca	Fe		K	Ca	Fe		K	Ca
Date 9/7/47 Fe Level	HCl Extract	Morgan's Extract			HCl Extract	Morgan's Extract			HCl Extract	Morgan's Extract		
Fe ₁	1.00	1.50	0.05	1.46	1.67	1.50	0.24	—	2.50	1.00	0.67	20.0
Fe ₂	3.00	2.00	0.20	1.00	2.25	1.00	0.25	1.53	0.47	2.00	0.60	10.0
Fe ₃	1.75	1.33	3.30	1.65	0.83	1.50	0.80	—	0.67	0.75	1.00	5.00
Date 5/8/47												
Fe ₁	2.00	5.00	0.10	1.67	1.00	4.00	0.13	1.13	0.50	4.00	0.63	—
Fe ₂	0.40	2.50	0.25	1.50	1.00	4.00	0.30	0.90	1.00	2.25	2.00	—
Fe ₃	0.40	4.00	1.00	1.67	1.00	3.20	0.27	2.67	1.00	2.00	1.20	—

TABLE IV.

MINERAL COMPOSITION OF DRY MATTER (SAMPLED 15/8/47).

Treatment		K Level															
		K ₁						K ₂									
		p.p.m. Fe	p.p.m. Mn	%K	%P	%Ca	p.p.m. Fe	p.p.m. Mn	%K	%P	%Ca	p.p.m. Fe	p.p.m. Mn	%K			
Fe ₁ Level	Leaf Sampled																
Fe ₁	Old	114	519	0.49	0.76	4.59	250	214	0.68	0.54	5.25	153	340	4.17	0.25	4.76	
	Young	264	388	2.69	1.33	8.94	194	444	3.27	1.20	3.39	113	482	7.39	1.00	1.26	
Fe ₂	Old	159	240	0.30	0.59	3.60	167	54	0.47	0.51	4.08	147	444	5.84	0.23	3.05	
	Young	118	89.2	1.01	0.78	1.55	135	170	1.91	0.88	1.59	130	230	4.98	0.77	1.15	
Fe ₃	Old	228	250	0.58	0.58	2.43	190	256	0.47	0.42	3.55	264	1097	6.72	0.30	2.39	
	Young	162	194	1.20	0.71	1.17	171	215	1.71	0.70	2.31	177	283	5.17	0.60	0.80	
		/															

the K_1 and K_2 levels increased iron supply tended to decrease the potassium content, especially in the young leaves. At the K_3 level increased iron supply increased the potassium content. Increased supplies of potassium decreased the iron content of the young leaves at the Fe_1 level, whereas at the Fe_2 and Fe_3 levels the iron content increased. The trends for the old leaves were inconsistent.

Certain other trends were apparent. The phosphorus content diminished with increased supplies of either iron or potassium; more markedly in the old leaves with increased potassium, and in the young leaves with iron increments. Calcium contents decreased regularly with increased iron supply (with one slight exception). Increase of potassium from K_1 to the K_2 level invariably increased the calcium content; further increase from K_2 to K_3 decreased it. The manganese content of the young leaves increased with increased potassium supply; in the old leaves manganese varied inversely with the iron content as the potassium supply increased.

The old to young leaf ratios of total nutrient content in dry matter are given in Table V. With the exception of the Fe_1K_1 treatment the old leaves contained slightly more iron than the young leaves and the old/young iron ratio showed surprisingly little variation. The old leaves contained less potassium than the young except in the treatments Fe_3K_2 and Fe_3K_3 where the reverse was the case. The ratio of old/young potassium increased regularly with increased iron supply.

With increased potassium supply the phosphorus ratio of old/young leaves declined, whereas increased iron supply consistently increased this ratio. Increased potassium supply was found generally to increase the old/young ratio for calcium content; a similar but less consistent tendency was observed for increased iron supply.

DISCUSSION.

Though the design of the experiments did not permit of the calculation of significant differences, the results most probably reflected real differences in the effects of the treatments, e.g. the total yield data. These results clearly indicated a Fe/K interaction effect. Total yield for the Fe_1K_3 treatment was about 36 times as great as that for Fe_1K_1 ; this could not be attributed to iron impurities in the high potassium nutrient as the iron content was found not to exceed 0.005 p.p.m. The iron content in dry matter of the young leaves was smaller and in the old leaves greater in the Fe_1K_3 treatment than for Fe_1K_1 . The absence of chlorosis and the general vigour of the Fe_1K_3 plants, however, indicated that here iron was apparently more available for chlorophyll formation. It seems likely from the leaf analyses and yield data that the Fe_1K_3 treatment resulted in the absorption of a greater total amount of iron during the growth period. Accordingly it appears that the potassium status of the nutrient governed, to some extent, the iron absorption capacity of the plant in addition to the efficiency of utilisation of iron in the plant.

The soluble iron results indicate that at the higher potassium levels the leaves contained more soluble iron than at the lower levels; this might imply that increased potassium status of the plant increased the iron availability. The mechanism by which these changes are brought about is obscure at present. It may be that increased potassium intake stimulates organic acid production and lowers the pH of the cell sap; it is known that plants with high tissue pH values have very low "soluble" iron and high total iron contents (9), (18). The visual effects and beneficial growth responses induced by increased

potassium supply at low iron levels were always accompanied by decreased phosphate content and it seems probable that one function of increased potassium was to diminish phosphate content of the foliage and thereby to increase the availability of iron within the plant. This point will be dealt with at length in a subsequent paper in this series. The seasonal trend in extractible iron suggests also that the proportion of iron effective in chlorophyll formation decreased with age as suggested by Jacobson (5).

Not only did potassium supply influence iron status, but increased iron supply decreased potassium content of the leaves at the lower potassium levels and at the same time delayed the occurrence of the potassium deficiency symptoms. The old/young leaf ratio for total potassium increased regularly with increased iron supply. Yet, in spite of the increased proportion of potassium in the older laminae, the potassium deficiency symptoms in the plant as a whole ultimately became more severe. It might be surmised, therefore, that at the high iron, low potassium treatments the presence of abundant iron impeded the full utilisation of the potassium present or restricted the translocation of potassium from old to young leaves. This would account for the earlier appearance of first symptoms of potassium deficiency in the Fe_1 series. The mechanism of these reciprocal effects of iron and potassium on the availability of these elements and the growth and composition of the plants, and their possible connection with the water relations of the leaf, will be the subject of further work.

SUMMARY

1. Potato plants (variety Majestic) were grown in sand culture with three levels of iron and three levels of potassium to produce deficiency, normal, and luxury consumption or excess effects of each element.
2. At the lowest iron and potassium levels the plants showed marked iron deficiency chlorosis; increased potassium supply decreased the severity of the chlorosis and gave tall, vigorous, pale green plants.
3. At the lowest potassium level, plants in all iron treatments showed severe potassium deficiency symptoms. These commenced in the older leaves, appeared first in the low iron treatments and were correspondingly delayed with increase in the iron concentration of the nutrient.
4. Increased iron concentration, however, increased the ultimate severity of the potassium deficiency symptoms, which became especially marked in the young shoot regions of plants.
5. Total yield and tuber weights increased regularly with the concentration of iron and potassium in the nutrient.
6. Increased potassium supply increased the total potassium content of the leaves and the old/young leaf ratio of potassium extracted in Morgan's reagent.
7. Increased iron supply usually decreased the potassium content of the young leaves. The old/young ratios for extractible and total potassium content of leaves increased with increased iron supply.
8. Increase of potassium supply generally increased iron content at the higher iron levels; at the lowest iron level the iron content decreased for the young and increased for the old leaves.
9. Total phosphorus content diminished with increased supplies of either potassium or iron; calcium content decreased with increased iron supply.

10. The experimental results support the idea that iron and potassium are interrelated in the metabolism of the potato plant and the reciprocal effects of this interrelationship on iron utilisation and potassium translocation are discussed.

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MOLYBDENUM AS A PLANT NUTRIENT.

II. EFFECT OF MOLYBDENUM DEFICIENCY ON SOME BRASSICA CROPS

By E. J. HEWITT and E. W. JONES.

The economic importance of molybdenum deficiency under field conditions is rapidly becoming recognised (1), (7), (8), (12), (14), (15). Some marked responses to molybdenum application in "whiptail" affected cauliflower and broccoli crops have already been obtained in this country (9), also in Australia (3, 13) and New Zealand (2). The technique developed at Long Ashton for the production of molybdenum deficiency symptoms in sand culture has now reached a stage at which it is possible to reproduce consistently these symptoms for various crop plants. Previous papers (5, 6) have indicated the nature of the problems of technique involved and also described symptoms for several different crops. It was shown that the few *Brassica* crops tested were particularly susceptible to molybdenum deficiency. Sensitive indicator crops are needed not only for field experiments but for physiological studies on nitrate metabolism and the genus *Brassica* was selected first for systematic experiments as it satisfies these requirements and presents in addition problems of special interest in relation to the condition known as whiptail.

EXPERIMENTAL.

Pyrex glass containers, sand, water and nutrient reagents similar to those previously described (6) were again used but with certain important modifications.

The sand was purified in batches in the automatic apparatus already described. Each batch was treated with separate charges of a mixture which contained 14% hydrochloric acid (A.R. grade) and 1% oxalic acid (technical grade) for three successive seven-hour periods. The available molybdenum content of the sand was reduced from 0.0003—0.0008 p.p.m. to 0.00002 p.p.m., after the final leaching with glass distilled water. The available molybdenum content was steadily reduced after each "run." The molybdenum content of the glass distilled water used for molybdenum deficient cultures was determined by bio-assay with *Aspergillus niger* (4) and found to be 0.000003 p.p.m.

The nutrient reagents were made up to give a nutrient in the following proportions:—(concentrations expressed as milliequivalents per litre) Ca^{++} 8.0; K^+ 3.0; Na^+ 1.33; Mg^{++} 3.0; NO_3^- 11; PO_4^{--} 4; SO_4^{--} 3.0; Fe^{+++} (as citrate) 0.3; the following micronutrients were also added (concentrations in p.p.m.) B 0.55; Mn 0.55; Cu 0.064; Zn 0.065; Mo 0.019; Co 0.003; Ni 0.003; Al 0.005; Ga 0.005.

The major nutrient reagents were purified by the copper sulphide coprecipitation method employed by Stout and Meagher (11). This method, when tested with *Aspergillus niger*, was found to be simpler and even more efficient than the 8 hydroxyquinoline method introduced by us in the previous two years. Solutions of each major nutrient reagent were prepared such that the requisite weight of recrystallised salt for two litres of solution was dissolved in about 1,700 ml. of glass distilled water. To this concentrated stock solution 20 ml. of a solution containing 5,000 p.p.m. of copper as copper sulphate



Fig. 1. Potato plants receiving 0.005 p.p.m. of iron but different levels of potassium. Left—high potassium; centre—low potassium; right—medium potassium. Higher levels of potassium increased growth and decreased the effects of iron deficiency chlorosis. With the highest potassium treatment the older leaves were paler than the young.



Fig. 2. Effect of molybdenum deficiency. Rape—Molybdenum omitted. Note mottling followed by marginal withering and leaf collapse.



Fig. 1. Effect of molybdenum deficiency. Brussels sprout—Molybdenum omitted. Note pronounced leaf cupping, marginal wilt, inrolling and leaf collapse (oldest leaves less severely affected than middle leaves).



Fig. 2. Effect of molybdenum deficiency. Hungrygap kale. Left: molybdenum added. Right: molybdenum omitted; note withering of leaves.

(A.R. grade) was added. Hydrogen sulphide generated in a Kipp's apparatus was bubbled rapidly through the stock solution for 30 minutes. The heavy precipitate of copper sulphide obtained was allowed to settle overnight. The solution was filtered, heated to remove residual hydrogen sulphide and cooled—after which it was generally necessary to refilter. Finally hydrogen sulphide was passed through a small aliquot of the solution to test for any residual copper sulphate; if detected the whole process was repeated until no more remained. The clear filtrate was made up to approximately 2 litres, ready for use. This procedure usually gave no difficulty with the nutrients used in this experiment, but for alkaline nutrient reagents, for example calcium nitrate, it was necessary to acidify the solution slightly to avoid repeated precipitations. The method employed in the preparation of molybdenum-free ferric citrate has been described (6) with the exception that after two recrystallisations the citric acid used was further purified by the copper sulphide co-precipitation method. The micronutrients were A.R. grade reagents and were not purified further.

The crops examined were :—cauliflower (Majestic), hungrygap kale, marrow-stem kale, rape (black), savoy cabbage, Brussels sprout, swede (purple top). All seeds (except swede) were sown on glass wool in molybdenum-free nutrient solution and transplanted to their respective beakers on 8/6/49 (14 days after sowing) and also sown directly in the pots. It became necessary to thin out the control cultures to one plant in each beaker (except swede, where three plants were left). Later in the season healthy cauliflower seedlings, not deprived of molybdenum, were transplanted to molybdenum deficient cultures in an attempt to induce whiptail effects.

RESULTS.

The molybdenum deficiency symptoms observed are described in the Appendix. Some yield and mortality data are given in Table I.

TABLE I.
EFFECT OF MOLYBDENUM DEFICIENCY ON DRY WEIGHT YIELDS AND MORTALITY OF
BRASSICAE IN SAND CULTURE.

Crop	Treatment	Dry wt. in gms. per plant at harvest (mid September)	—Mo/+ Mo yield ratio as per cent	Approx. per cent killed	
				19/7/49	7/8/49
Swede	+ Mo — Mo	not recorded; all deficient seedlings killed by August 18th		40	90
Sprout	+ Mo — Mo	66.5 (tops) 0.2 "	0.3	10	25
Cauliflower ..	+ Mo — Mo	187.8 (whole plant) 1.25 " "	0.7	15	20
Rape	+ Mo — Mo	45.7 (tops) 0.56 "	1.2	0	0
Hungrygap kale	+ Mo — Mo	132.3 (whole plant) 4.30 " "	3.25	21	42
Marrowstem kale	+ Mo — Mo	219.7 (whole plant) 17.34 " "	7.9	0	14
Savoy cabbage ..	+ Mo — Mo	70.25 (whole plant) 17.0 " "	24.2	7	7

The different varieties generally showed marked similarity in visual effects. The symptoms usually commenced in the mid-stem expanding leaves as a pale yellow, diffuse, intervenal mottling which was accompanied by a grey-green coloured marginal limpness or wilt (Plate V, Fig. 2). This stage was followed or sometimes preceded by a distinct cupping of the lamina, with the exception of hungrygap kale and rape where no cupping was observed. The marginal leaf area became scorched and finally the whole lamina, in severe instances, was buff coloured and completely withered. Collapse of the lamina was usually sudden and occurred either at the point where lamina adjoined petiole or sometimes at the apex end of the lamina itself (Plate VI, Fig. 1). If leaf collapse was severe death of the growing point followed. This accounted for the relatively high mortality in swede and sprout. The cotyledons did not always remain dark green for so long as noted on previous occasions (5) (6) but in several instances became yellow (hungrygap kale) or sometimes pale orange (sprout); this perhaps might be attributed to the improved technique and consequent greater demands on the cotyledonary reserves, or possibly to the exceptionally high light intensity and temperatures that prevailed. In all crops molybdenum deficiency symptoms were readily produced in the first two to four weeks after planting or sowing; where excessively severe, as in swede, the seedlings were killed outright in four to five weeks. Molybdenum deficiency drastically retarded growth (Plate VI, Fig. 2) and kept the plants in a dwarfed seedling condition. Rape plants were almost rosette in habit (Plate V, Fig. 2), with very short internodes as compared with the controls. The kales and savoy cabbage showed a tendency to recover.

The attempts to reproduce whiptail in cauliflower artificially in sand culture were less striking than in 1947 (5). Symptoms appeared in the young or middle leaves some eight weeks after transplanting the young cauliflower plants. The growing point became vestigial after browning of the youngest leaves which remained stunted. Restricted laminae with wavy margins and bare stunted petioles, followed by death of the growing point, also occurred after partial recovery in hungrygap and marrowstem kales grown with continued limitation of the molybdenum supply. These effects were all analogous to the condition of whiptail in the field except that the capacity for continued extension of young petioles practically devoid of laminae was absent.

The individual varieties varied in their susceptibility to molybdenum deficiency. The general order of susceptibility judged visually and from yield and mortality data (Table I) was concluded to be: swede, sprout, hungrygap kale, cauliflower, rape, marrowstem kale and savoy cabbage. In the early stages savoy seemed to be quite as susceptible as rape but later appeared to recover more readily, as also did marrowstem kale.

DISCUSSION.

Comparison of the times required for the appearance of deficiency effects in these and earlier experiments indicate a general improvement in technique. The failure to prevent completely the recovery of certain varieties at a later stage of growth, however, raises certain points of interest. Unpublished experiments on sand purification, and examination of samples by means of an *Aspergillus niger* method, indicate that the removal of molybdenum by acid treatment may be slow and not complete even after three "runs." Use of dilute alkali on the other hand has also been tested and appears to be especially promising. The recovery of certain varieties may thus possibly be related to the change in pH of the nutrient from acid to alkaline that occurs rapidly

in contact with the roots. It also appeared from the data on root weights that the greatest recovery was usually shown by plants with the greatest root production. The effects of pH change and root surface area would be additive.

The genus *Brassica* presents features of special interest in relation to the distribution of visual effects of molybdenum deficiency. No other crops studied up to the present show the same sensitivity in the growing point or young foliage. The abnormal effects produced in these regions appear to occur most readily either after partial recovery or when young healthy plants are transplanted to a culture deprived of molybdenum. Under these circumstances, the younger parts are usually most affected and old leaves may be normal or almost so. This response has been consistently produced with cauliflower, swede, marrowstem kale and hungrygap kale on two or three separate occasions during the last three years. All other crops (for example, tomato, legumes, cereals and beet) have shown effects in old and middle leaves but not in the growing point.

The significance of these effects in the development of the condition known as whiptail is evident, but the origin of certain features of the disorder in the field is still obscure. Numerous effects have been recorded in field plants where whiptail has been diagnosed (9). These effects include death of growing point, weak or abnormal curd, wavy leaf margins, malformed stunted or vestigial leaves, or abnormally elongated petioles, often bare or with a narrow strip of lamina, which have led to the use of the name whiptail. Most of these effects have been recorded on different occasions in the molybdenum deficient sand culture plants already described. One feature, however, namely the rapid elongation of bare petioles or foliage with exceptionally narrow laminae, has been only rarely observed in the sand culture plants (5) and may be less common in the field than the other effects mentioned above.

It is becoming increasingly obvious that where molybdenum is deficient the mechanism (possibly hormonal in nature) that controls leaf organization in *Brassicae* is upset. The possible relation of this effect to the accumulation of unassimilated nitrate and to abnormal nitrogen metabolism requires further investigation. It is relevant also to point out here that molybdenum may function not only in the presence of nitrate or nitrite but appears also to influence metabolic systems in the absence of these compounds (unpublished data 1949). It is logical therefore to consider the possibility that the cause of the leaf deformation may lie in the accumulation or depletion of other substrates involved in these reactions. The nature of these reactions, and of their relationship to nitrate assimilation, is still obscure. These problems comprise the subject of continued experiments.

SUMMARY.

1. The following *Brassica* crops were grown in molybdenum deficient sand culture: cauliflower, hungrygap kale, marrowstem kale, rape, savoy cabbage, Brussels sprout, swede.
2. An improved technique for nutrient reagent purification, involving co-precipitation of molybdenum impurities with copper sulphide, was used. The purity of sand, water and reagents was checked by means of an *Aspergillus niger* bioassay technique.
3. All crops showed severe molybdenum deficiency symptoms, especially in the seedling stage; yields were reduced to 10% or less (except savoy) and mortality was high.
4. The general order of susceptibility to molybdenum deficiency, as

judged visually and from yield and mortality data, was :—swede, sprout, kale (hungrygap), cauliflower, rape, kale (marrowstem), savoy cabbage.

5. The ability of certain varieties to recover partially from the effects of molybdenum deficiency, and the similarity of later symptoms to the whiptail condition in cauliflower, are discussed in relation to the special effects noted in *Brassica* crops.

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APPENDIX.

Visual Symptoms of Molybdenum Deficiency.

The approximate time, in days after planting or sowing, necessary for the development of molybdenum deficiency symptoms under the conditions of the experiment is given in brackets.

Cauliflower (cf. also reference 5)

Growth markedly reduced ; marginal wilt of middle leaves with pale yellow-green intervenal mottling (18 days) followed by rapid incurling or cupping of margins and shrivelling or complete scorching of older leaves (25 days). Sometimes complete death of growing point. Cotyledons remained dark green after appearance of symptoms. Seedlings transplanted to molybdenum deficient cultures at the third leaf stage showed leaf mottling about 4 weeks later, followed by distortion, stunting, browning of young leaves and death of the growing point.

Kale (hungrygap)

Growth dwarfed. Very pale green foliage with intervenal diffuse yellow mottle of middle expanding leaves (10 days). No distinct cupping. Leaf margins pale or yellow, becoming limp, wilting and withering. Laminae completely scorched, leaving green petioles (20 days). Cotyledons yellowed. After partial recovery prolonged deficiency at later stages in life cycle produced effects in young foliage; youngest and expanding leaves had narrow distorted laminae, were brown tipped and had stunted or partly elongated petioles.

Kale (marrowstem) (cf. also reference 6).

Growth stunted; pale grey-green foliage; expanding leaves (middle and younger) with yellow green margins, forward cupping and bleached out regions at leaf tips (10 days). Oldest leaves developed buff coloured marginal necrosis and collapsed suddenly (20 days). This was followed by rapid scorching and bleaching of intervenal region to a buff colour (28 days). Cotyledons yellow green. Growing point sometimes killed (14 weeks), usually after partial temporary recovery.

Rape

Growth stunted; vigour markedly reduced. Foliage pale green; pronounced yellow mottling of lamina (9 days), no leaf cupping. Buff coloured or dull yellow bleaching of leaf margins of middle leaves (19 days). Complete withering of older laminae (28 days). Later, some plants killed by severe deficiency.

Savoy cabbage (cf. also reference 5).

Growth dwarfed; marked intervenal mottling and yellowing of leaf margins; forward cupping. Watersoaked marginal areas followed by buff coloured scorch and collapse of leaf at tip of middle leaves (10 days). Youngest leaves showed inrolling (28 days). Many older leaves less affected.

Brussels sprout

Growth markedly reduced, grey green, afterwards pale yellow green generally; slight diffuse intervenal mottling and very pronounced regular forward cupping of middle and expanding leaves (9 days). Marginal chlorosis, lamina becoming water soaked and membranous inwardly from tip. Ultimately lamina completely cream or buff coloured. Collapse of leaves from petiole, wilting of old leaves (28 days). Cotyledons losing green colour, becoming orange. Death of growing point (10-14 weeks); many plants killed.

Swede (cf. also reference 6).

Growth very restricted; all seedlings with pronounced yellow green tint and diffuse mottling (13 days after emergence); buff coloured leaf scorch and withering from tip of first leaf. Inrolling of lamina in younger leaves followed by marginal scorch, ultimate collapse and withering of laminae (21 days) and early death of all seedlings (25-35 days after emergence).

VIRUS DISEASES OF FRUIT TREES

I. SOME FIELD OBSERVATIONS ON VIRUS-LIKE SYMPTOMS ON STONE FRUIT IN SOUTHERN ENGLAND

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From time to time virus-like symptoms have been reported on top-fruits in the United Kingdom and, in some cases, their virus origin has been demonstrated*, but until recently there has been little or no systematic investigation of this important problem and its possible significance for the fruit industry of the British Isles. During the summer of 1949, while the author was stationed in exchange at Long Ashton, representative cherry and plum orchards in Kent, Somerset, Herefordshire and Worcestershire were carefully inspected and, in a few instances, charted to obtain a preliminary estimate of the occurrence, type and distribution of symptoms of possible virus infections in these crops. Because long incubation periods are normally required for the tree-fruit viruses, and because adequate supplies of suitable test plants were not available, it was impossible in the course of a single year to undertake large-scale transmission experiments or even to obtain satisfactory results from the indexing that was done. This report is therefore concerned primarily with field observations and is of necessity somewhat speculative in character.

Among the symptoms elsewhere associated with virus infection, the most reliable are various colour patterns, chiefly variants of green or yellow shades more or less irregularly distributed over the leaf surface. These include spots and rings of various types and sizes, line and "oak leaf" patterns, vein-banding and vein-clearing, mosaic mottling and general chlorosis. Even some of these, for example certain types of spots, mottles and vein-banding, may be simulated by the effects of insect infestation, which, however, can usually be detected by the presence of the insect or its remains. On the whole, the foliage markings of virus origin are readily distinguishable from those due to mineral deficiencies, but, in some cases, there may be a certain amount of overlapping of symptom type as well as of interaction. Diagnosis by symptom alone therefore is not always possible. Other symptoms, such as necrotic spotting, shot-holing, laceration and distortion, bud failure, phloem or bark necrosis, die-back, stunting, rosetting, etc., may arise from a variety of causes and are not necessarily diagnostic of virus infection unless accompanied by characteristic colour patterns and/or verified as part of the symptom picture by carefully controlled transmission experiments. As far as possible, these obscuring factors have been taken into consideration in assaying the field observations reported below. Obviously, though certain similarities in symptom expression are pointed out, no attempt can yet be made to identify any of the disorders observed here with the viruses known to occur in North America and elsewhere, until a good deal of comparative experimental work has been done.

* Dr. I. W. Prentice (4) while working at East Malling Research Station succeeded in graft-transmitting two viruses of cherry; (i) the disease described herein on Ronald's Black, and (ii) a mosaic of Early Rivers. He also infected almond with a line-pattern disease from peach.

Symptoms Observed in Cherries (*Prunus avium*).

A wide range of virus-like symptoms were observed in each of the many cherry orchards visited. In the two orchards systematically surveyed and charted, between 40 and 60 per cent of the trees appeared to be virus infected. In several instances, affected trees of a given variety exhibited the same type of symptom. A few examples are cited.

(i) In one Herefordshire orchard, most of the trees of the variety Frogmore were characterised by long and abnormally narrow leaves, many of which were more or less mottled and had some irregular large necrotic spots scattered over them. The vigour of the trees appeared to have been impaired and a considerable proportion of the shoots had either died back or were bare of leaves where lateral buds had failed to leaf out. The crop appeared to be lighter than normal.

(ii) In the same orchard, the variety Noble showed die-back and bare lengths of shoot caused by the death and gummosis of buds and spurs. The leaves tended to roll upwards, parallel with and towards the mid-rib, but had no abnormal markings. The owner reported that this condition was an annual recurrence.

(iii) A somewhat similar gummosis of buds was observed in a North Somerset nursery on the varieties Merton Heart, Merton Bounty and Merton Favourite. The early leaves on affected trees showed intervenal chlorotic and red spotting, followed by necrosis and laceration, varying somewhat in detail and severity from variety to variety.

(iv) In the variety Ronald's Black, in an orchard at Cranbrook, Kent, the leaves were narrow and asymmetrical, with irregular margins. They were also marked with irregular line and mosaic patterns and some necrotic spotting. Shoot internodes tended to be short and the fruit to be pointed and withered. Here, the aberrations in leaf shape resemble the disorder known in America as "crinkle," which so far has not been transmissible, but the other symptoms would suggest virosis.

(v) At Owen's Court, Kent, a number of trees of the variety Napoleon, about 25 to 30 years old, showed pronounced mottle and small ring patterns on their leaves and were reported by the owner to have made little growth and to have been relatively unproductive for several years.

In the above-mentioned cases, it is possible to regard differences in symptoms as different varietal reactions to a single virus. On the other hand, evidence for the occurrence of several viruses is to be found in a comparison of symptoms on individual trees of a single variety. For example, the following array of symptom types has been observed on different trees of the variety Early Rivers. With this variety, the range of symptoms was likely to be as wide in a given orchard or locality as in widely separated areas.

Type 1 : Severe necrotic spotting, shot-holing and tattering, accompanied by ring and line patterns, on the leaves produced early in the growing season. Later leaves were more or less normal, and the trees appeared to "recover" by mid-summer. The fruit tended to be small and to ripen unevenly.

Type 2 : Leaves somewhat narrow and marked with a fine mosaic or ring pattern, but having little or no necrotic spotting.

Type 3 : Conspicuous large, coarse, pale green rings and lines, due to vein-clearing in localised areas.

Type 4 : On some leaves, a fine white speckling or mosaic over the whole leaf surface, somewhat resembling the type of marking associated with "crinkle," but without the characteristic marginal distortion ; on other leaves,

large, isolated and fairly conspicuous pale green rings, and slight necrotic spotting. It seems likely that these are symptoms of two disorders in one tree.

Type 5: Leaves faintly marked with large but finely textured greenish ring and line patterns, usually occurring singly.

Type 6: Ring and line patterns usually coarse but well defined, ranging in colour from yellowish green, through yellow to reddish brown, and accompanied by slight necrosis.

These various symptom types have also been observed on other cherry varieties. Another widely distributed symptom not definitely associated with any one variety was an irregular, white, marginal mosaic pattern of the "crinkle" type, occurring on leaves scattered over affected trees.

A disorder characterised by leaf-distorting enations, like those of the rasp-leaf virosis of the Pacific North-West, has been discovered in at least two districts of Kent. A number of different varieties are affected.

Symptoms Observed on Plums.

On the whole, most plum varieties appear to be more or less tolerant to virus infection and, with a few notable exceptions, symptoms tend to be either marked or obscure. Even in what promises to be a disease of considerable importance in this country, the recently discovered "plum stunt" (Crowdy and Luckwill, 1950), diagnostic symptoms are tantalizingly vague. Diagnosis of virus diseases of plums by visual inspection, then, is not usually satisfactory, unless supported by indexing techniques. Consequently, it is to be expected that virus diseases are more prevalent in plums than the present record shows, and that tolerant plum varieties are dangerous potential sources of infection for more susceptible varieties and species of *Prunus*.

Among the characteristic leaf markings and other possible indications of virosis to be seen in most plum orchards were:

(i) Pale green or yellowish green fine lines, sometimes forming irregular "oak leaf" patterns but more frequently rings and irregular patterns lying usually between but sometimes along the larger veins. This condition is of frequent occurrence on Yellow Pershore, but has also been observed on Evesham Wonder, Giant Prune, Pond's Seedling, Purple Pershore, Rivers Early and Victoria.

(ii) Variants of this pattern in the form of irregularly distributed vein-clearing and diffuse mosaic on Pond's Seedling, Purple Pershore and Wye Cross.

(iii) A striking, coarse, yellow, banded chlorosis, appearing occasionally as broad lines but more frequently as "oak-leaf" patterns in the varieties Czar, Diamond, Oullin's Golden Gage, Pond's Seedling, Rivers Early, Victoria, Warwickshire Drooper and Wye Cross. This condition seems to be distinct from the preceding ones and bears closer resemblance to the line-pattern virosis as described in North America.

(iv) Mottling and an irregular, blotchy chlorosis, accompanied by premature leaf drop early in the growing season, were common on the variety Monarch in a North Somerset plantation. Affected trees were markedly spindling and straggling in growth habit.

(v) A yellow mosaic affected at least one Belle de Louvain tree in an orchard in Kent. The resemblance of this symptom to apple mosaic may be coincidental, but the fact that this tree was growing next to a mosaic apple tree suggests the possibility of a common cause.

Symptoms observed on Peaches.

Observations on peaches were comparatively few, but it is of interest to note that in several glass houses, where peaches were growing on plum root-stocks, several types of line, ring and mosaic patterns were fairly common. These disorders appeared to have little ill-effect on growth or crop, but it is quite possible that more serious virus troubles could be introduced through diseased root-stocks.

Discussion.

This brief survey leaves little doubt that virus diseases are more prevalent on stone fruits in Great Britain than is generally realised. While it may be argued that these troubles are not as spectacular and devastating as some that have appeared elsewhere, they cannot be dismissed as of minor economic importance as long as their extent and their effects are so little known. In the first place, a virus may seriously affect only a few varieties and have but little effect on the rest, for example, prune dwarf (5) or rubbery wood of apple (3). In the second place, a virosis which might be classed as mild with respect to its general symptoms, may be severe in its influence on yield, as for example, some mild strains of cacao virus (2). Again, unpublished data from experiments in progress in Ontario indicate that, with some cherry viroses, significant reductions in yield can best be detected in the cumulative records of the total crop produced over a number of years.

It is extremely probable, too, that some serious disorders, for example, those reported in the Merton cherry varieties and on Noble, may actually be of virus origin, but, in the past, have been attributed to bacterial infection or some other cause. There is also good reason to suspect that at least some of the incompatibility phenomena are manifestations of virosis. If that is the case, then the whole problem of root-stocks stands in need of review. Certainly the danger of contamination of clonal root-stocks, scion sources and new varieties would be minimised if the virus factor were taken into account and suitable procedures were adopted to establish and maintain virus-free sources of propagating material. The practice of top-working non-productive trees should also be discouraged unless steps are taken to ascertain that a virus is not involved.

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VIRUS DISEASES OF FRUIT TREES

II. OBSERVATIONS ON RUBBERY WOOD, CHAT FRUIT, AND MOSAIC IN APPLES.

PROGRESS REPORT.

By L. C. LUCKWILL and S. H. CROWDY.

INTRODUCTION.

In 1944 a number of short articles were published (6, 11, 12) in which attention was drawn to certain abnormalities of the apple variety Lord Lambourne. These were referred to under the names "rubbery wood," "chat fruit," "mottle" or "mosaic" and "flat limb," and it was suggested that they were in the nature of virus diseases. Certain of these abnormalities have also been recorded on other varieties of apple.

In view of the economic importance of Lord Lambourne to fruit growers in this country, the wide distribution of these diseases, and the serious loss of crop which certain of them can cause, further investigations were undertaken into their nature, origin and method of distribution. The ultimate object of these investigations was to discover if and how it might be possible to raise healthy clones of this valuable apple and to maintain them free of disease.

The work was started at Long Ashton in 1944 by Dr. T. Swarbrick, who was responsible for the collection of most of the data on the origin of the diseases and who carried out the original surveys of the orchards. Since 1945 the work has been continued and extended by the present authors and in this article the results obtained up to the end of 1949 are reported.

DESCRIPTIONS OF THE DISEASE.

Rubbery Wood.

Host range. In addition to Lord Lambourne, rubbery wood has been recorded on the following varieties: James Grieve (the female parent of Lord Lambourne); Miller's Seedling (1); Dartmouth Crab (1); Kingston Black; Sturmer Pippin. On none of these varieties, however, is the disease as widespread or as serious as on Lord Lambourne.

Symptoms. In trees affected with rubbery wood some or all of the branches lack their usual rigidity, and wood one inch or more in diameter may often be readily bent with the fingers of one hand. In Lord Lambourne the normal spreading growth of the variety is replaced by a very characteristic drooping habit (Plate VII, Figs. 1a and 1b). The internodes are shorter than normal, the amount of annual growth is much reduced, and, in severe cases, the whole tree is extremely stunted. Rubbery trees may carry normal fruit, but the total yield is much reduced owing to the small size of the trees.

The flexible nature of the branches is due to incomplete lignification of certain of the woody elements, particularly the fibres, which are largely responsible for the strength of the wood. The vessels too, often fail to lignify and sometimes collapse. This lack of lignification is most severe in the summer wood, but in very rubbery branches areas of spring-formed wood may also remain unligified (1).

The rubbery condition first becomes marked in second year growth and abnormally lignified wood may continue to be formed for five or more years, after which normal wood may sometimes again be produced. When this happens the branch eventually becomes rigid and the drooping habit is fixed.

Chat Fruit.

Host range. This disease has so far been recorded only on Lord Lambourne. In England it seems to be prevalent wherever this variety is grown.

Symptoms. Economically this is the most serious of the diseases under consideration, as the entire crop from chat-fruited trees may be unmarketable. In severe cases the fruits remain green, or green with a dull red flush, and do not exceed $1\frac{1}{2}$ —2 inches in diameter at maturity. In less severe infections the fruit may attain almost normal size, but fails to develop its characteristic bright red colour, remaining a dull brownish red. Severely chatted trees are exceptionally upright and vigorous in growth, and may often be recognised by this character alone. This disease occurs independently of rubbery wood, though both troubles may be shown by the same tree.

Mosaic.

Host range. Apple mosaic appears to have a much wider host range than either of the above diseases, and most varieties of apple seem susceptible. In this country very severe infestations have been seen on Lord Lambourne, Bramley's Seedling, and Worcester Pearmain, and somewhat milder symptoms on Edward VII, Crawley Beauty, Allington Pippin, James Grieve, Newton Wonder, and on the Malling clonal rootstocks. Some indication has been obtained that certain varieties, such as Lord Derby, may be symptomless carriers of mosaic.

Symptoms. This disease is characterised by the appearance of irregular chlorotic areas on the leaves which develop either around the veins and veinlets, giving a reticulate pattern on the leaf, or, more frequently, in the interveinal areas, giving an irregular mottled pattern. The chlorotic areas are at first creamy white in colour but as the season advances they become necrotic and fall out, and later the whole leaf is shed, causing a premature defoliation of the tree. The disease is thought to be identical with the *Pyrus virus 2* described by Smith (9).

The severity of the symptoms in mosaic infected trees varies somewhat from season to season, being most pronounced in dry summers such as that of 1949. In mild cases of mosaic infection the trees, if they are free from rubbery wood and chat fruit, may grow and crop normally, but in more severe cases the lack of chlorophyll and early defoliation cause a very marked weakening of the tree and must reduce the crop very considerably.

Flat Limb.

Host range. Flat limb is a prevalent disease of Gravenstein in all countries where that variety is grown. In this country typical Gravenstein flat limb has been seen on the variety Labygard (a Gravenstein type) in the National Fruit Trials at Wisley. A different type of flat limb, which appears to be of little economic importance, is of almost universal occurrence on Lord Lambourne trees in the west of England, but is of less frequent occurrence in the eastern counties.

Symptoms. In Gravenstein trees infected with flat limb the branches show a much twisted and contorted ribbon-like growth. Flat limb in Lord

Lambourne is usually confined to the lower parts of the main scaffold branches and is not shown by the younger wood. In contrast with Gravenstein the flattening is always in the vertical plane and there is no twisting or contortion of the branches. Flat limb, similar to that shown by Lord Lambourne, has been observed in trees of a number of other varieties, but it is not yet known whether it is the same disease as occurs in Gravenstein.

HISTORY OF THE DISEASES.

The variety Lord Lambourne was raised by Messrs. Laxton Bros. of Bedford about 35 years ago from a cross of James Grieve with Worcester Pearmain. In 1922 it was awarded the R.H.S. cup for the best seedling apple of the year and in 1925 was entered in the National Fruit Trials at Wisley. Unfortunately, the original tree is now no longer in existence, but there seems no doubt whatever that it was completely free from chat fruit, rubbery wood and mosaic.

Scions from the seedling tree were worked on to Malling II and, in 1927, 170 of these two-year-old trees were planted on a farm in Worcestershire. All of them made excellent growth and have consistently borne good crops of first quality fruit. Three complete rows of these trees are still in existence and have been kept under close observation since 1944. Within recent years a small percentage of these trees have developed slight symptoms of rubbery wood and chat fruit, but the vast majority of these trees, now 24 years old, can still be described as completely healthy. Selected trees of this batch, hereafter referred to as the "original trees," have provided a reliable source of healthy Lambourne scions for use in the grafting experiments to be described later.

TABLE I.
ANALYSIS OF SURVEY OF "ORIGINAL" TREES OF LORD LAMBOURNE (BUDED 1925).

Condition of tree				No. of trees showing symptoms	
				1944	1948
Completely healthy	..	..	..	145	134
Rubbery	{	Suspected		8	8
		Definite		0	8
Chatted	{	Suspected		0	2
		Definite		0	1
Total No. of trees	..	..	..	153	153

From 1929 onwards, as a result of the increasing popularity of Lord Lambourne, this variety was used extensively for the headworking of older and less popular varieties. For the earliest headworkings grafting material was taken exclusively from the "original" trees, so that there is no doubt that the scions used were free from rubbery wood, chat fruit and mosaic. Nevertheless many of the headworked trees developed severe symptoms of one or more of these troubles within 2 to 3 years of grafting. The records of four such headworkings are summarised in Table II, from which it will be noted that only that on Redcoat Grieve was successful. It is of interest to note

that Crane (6) records an instance where healthy Lambourne top-worked on Redcoat Grieve developed chat fruit and similar scion material worked on Excelsior developed rubbery wood.

TABLE II.

RECORDS OF SOME OF THE EARLIEST HEADWORKINGS WITH LORD LAMBOURNE. THE SCION WOOD IN ALL CASES WAS COLLECTED EXCLUSIVELY FROM THE "ORIGINAL" TREES WHICH WERE FREE FROM RUBBERY WOOD AND CHAT FRUIT.

Date of Working	Variety worked	No. of trees	Results
1929	Unknown	26	Of the 14 trees remaining in 1949 all were rubbery and 5 were also chat fruited. In addition 4 showed mosaic.
1932	Lady Sudeley	8	Nearly all these trees are stated to have gone rubbery within 2-3 years of grafting. Of the 3 trees remaining in 1944 all were rubbery and the fruit on 2 was chatted.
1933	Redcoat Grieve	120	No symptoms of rubbery wood or chat fruit developed on these trees. In 1944 54 trees remained and all were still healthy.
1933	Delicious	30	80-90% of these trees are stated to have gone rubbery within 3 years of grafting. Examined in 1944 only 7 trees remained. Of these 6 were rubbery and 1 was chat fruited.

There are many other records of Lord Lambourne having failed when top-worked on to other varieties, but in most cases it is not possible to be certain that the original scion material was healthy: often it was collected indiscriminately from previously headworked trees, which may or may not have been free from disease, and no records were kept. It is clear that such evidence is of no scientific value.

From the records presented in Table II however, it is apparent that rubbery wood and chat fruit first occurred in the early 1930's, following the head-working of healthy scions of Lord Lambourne on to apparently healthy trees of other varieties. It also seems certain that these troubles appeared spontaneously and independently in different plantations at about the same date and that their origin cannot be traced to any one particular headworking. Once they had appeared, however, the indiscriminate collection of scion wood from infected trees undoubtedly contributed to their rapid spread, so that to-day there is scarcely a plantation of Lord Lambourne in the country which is entirely free from these diseases. Rubbery wood is also very prevalent in other countries where Lord Lambourne is grown, particularly in Norway, where the variety was first introduced to the pomological research station at Njøs as early as 1925. In 1927 there was a further introduction of Lord Lambourne from Messrs. Laxton Bros. to the Agricultural College of Ås and the main distribution in Norway appears to have taken place from these two centres.* Since, in 1927, rubbery wood had not yet been recorded, it seems that the disease must also have appeared independently in Norway.

Apple mosaic has been known for a much longer time than either chat

* From private correspondence with T. Ramshill, Norwegian Plant Pathological Institute, Oslo.

fruit or rubbery wood, and there is no indication as to how or where it first originated. According to Bradford and Joley (3) infectious variegation of apple foliage was recognised by Vibert in France as early as 1835, and it is now a common disease in all apple growing areas of the world.

EXPERIMENTAL DATA.

Graft Transmission.

In order to investigate further the nature of these diseases a number of grafting experiments have been carried out, the results of which are given below :

Rubbery wood

(1) In March 1945 ten 5-year old trees of Lord Lambourne showing pronounced symptoms of rubbery wood were headed back and grafted with scions of healthy Lambourne taken from the "original" trees. Five of these trees died, but the remaining five, when examined in the autumn of 1946, were all found to be extremely rubbery. Scions from the same source worked at the same time on to neighbouring non-rubbery trees were all making normal growth.

(2) In this experiment ten trees of Lord Lambourne which had been headworked on to Ontario were used. All the trees, to a greater or lesser degree, were rubbery. Two of the main scaffold branches on each tree were cut back in March 1948 and rind-grafted with healthy Lambourne scions from the "original" trees. On one branch of each tree the scions were placed directly on the Lambourne wood, but the other branch was cut back below the previous headworking so that the scions were grafted to the Ontario wood. Examined in the autumn of 1949 the majority of the scions were found to have made good growth but all were rubbery, irrespective of whether they were growing on Lambourne or Ontario wood.

(3) In the Spring of 1945 a healthy bush tree of Bramley, approximately 12 years old and having 5 main scaffold branches, was cut back and worked with Lambourne scions. Four of the main branches received healthy scions from the "original" trees but on the remaining branch rubbery scions were set. During the past four years there has been a progressive spread of the disease throughout the tree (Plate VII, Fig. 2). By 1949 even those Lambourne scions furthest from the point of infection showed symptoms of rubbery wood, but a Bramley shoot which was allowed to grow from branch 5 remained healthy.

These experiments have established beyond doubt the fact that rubbery wood is transmissible by grafting: it may therefore be classed as a virus disease. Further, it can be transmitted through varieties such as Ontario and Bramley's Seedling which do not show symptoms of the disease.

Chat Fruit.

Owing to the greater length of time taken for the symptoms to manifest themselves, the evidence for the graft transmissibility of chat fruit is not yet as extensive as that for rubbery wood. Nevertheless, as far as it goes, the evidence points strongly to the conclusion that this disease too is of a virus nature.

In March 1945 six severely chat-fruited trees of Lord Lambourne were cut back and headworked with healthy Lambourne scions from the "original" trees. Examined in September 1946 five of these trees were carrying fruit

which was very definitely chatted, and one tree was in addition slightly rubbery. The remaining tree had died. The orchard of which these trees were a part was grubbed during the following winter so that it has not been possible to continue observations. Further experiments on the transmission of chat fruit are now in progress at Long Ashton, however, and should yield results within the next two years.

Mosaic

The transmission by grafting of apple mosaic has been demonstrated by many workers (2, 3, 4, 5, 8, 10). These results have been confirmed at Long Ashton, but in view of the abundance of evidence as to the virus nature of this disease, will not be set out in detail here.

Spread in the Orchard.

A point of obvious practical importance in connection with these diseases is whether, in addition to being transmissible by grafting, they have a natural method of spread under field conditions. To gain information on this question a number of plantations of Lord Lambourne in two different localities have been surveyed at intervals since 1944, each tree being scored for rubbery wood, chat fruit and mosaic.

The chief difficulty in this work is how to decide, from the visual symptoms alone, whether a tree is infected with rubbery wood or chat fruit. In the early stages of the diseases the symptoms are often confined to a single branch and are by no means obvious. For this reason surveys have always been carried out by two observers and a tree has been classed as definitely rubbery or definitely chatted only when both agreed that it was so. Where opinions varied as to the status of the tree, or where both regarded it as a doubtful infection, the tree was scored as "suspected rubbery" or "suspected chat." The records are further complicated by the seasonal variation in symptoms, particularly in those of chat fruit. With mosaic the intensity of the symptoms varied from season to season, but no instance has yet been recorded of a mosaic infected tree showing an apparent recovery, and it is always possible to say definitely whether the tree has mosaic or not. Bearing in mind these limitations it will be clear that observations must extend over a number of years before definite conclusions can be drawn.

The records to date are given in Table III, from which it will be seen

TABLE III.

INCREASE IN THE NUMBER OF INFECTED TREES IN PLANTATIONS OF LORD LAMBOURNE
APPLES BETWEEN 1944 AND 1949.

Locality	No. of trees	Year	No. of trees showing symptoms.					
			Rubbery Wood		Chat Fruit		Mosaic	
			Definite	Suspected	Definite	Suspected	Definite	Suspected
1	167	1944	108	14	18	20	43	0
		1948	137	7	23	11	67	0
		1949	150	4	26	16	71	0
2	361	1944	35	14	139	74	0	0
		1946	74	7	159	74	0	0

that in both localities there has been a very definite and marked increase in the number of infected trees since the records were started in 1944. The increases are particularly striking for mosaic and rubbery wood and seem far too large to be accounted for by seasonal variations or observational errors. It is perhaps significant that these diseases are the most readily recognised. The records for chat fruit are less satisfactory, being complicated by the large proportion of trees in the "suspected" category, but here too, the figures suggest strongly that the disease is spreading.

Spread diagrams for apple mosaic in three different blocks of Lord Lambourne trees are given in *Fig. 1*. All these trees had been headworked

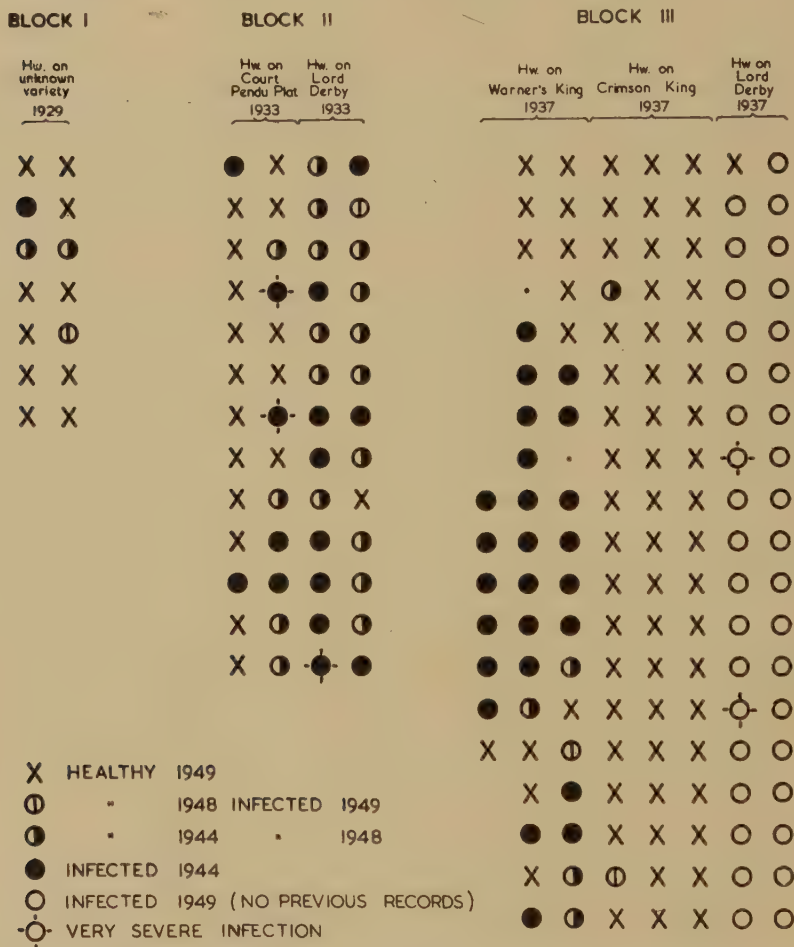


Fig. 1. Diagram showing the spread of apple mosaic between 1944 and 1949 in three separate blocks of Lord Lambourne trees, headworked on to different varieties.

on to other varieties as indicated in the diagram. The almost complete infection of Lambourne worked on Lord Derby and Warner's King suggests that many of these trees may already have been infected with mosaic before they were headworked and that the disease is now slowly spreading on to the Lambourne worked on other varieties. It is perhaps significant that, even on severely infected trees, the water-shoots from the Lord Derby and Warner's

King stems always appeared to be free of mosaic, which suggests that these varieties may prove to be symptomless carriers.

It is concluded from these records that, in addition to being transmissible by grafting, rubbery wood and mosaic spread naturally in the field, although the rate of spread appears to be slow. It is also considered probable, though not yet definitely established, that chat fruit too can spread naturally.

Regarding the mode of spread the data give little indication. Although new infections usually occur next to already infected trees the spread diagrams for mosaic given in *Fig. 1* show that this is not invariably so. This suggests that insects, rather than natural root grafting, may play a part in spreading this disease. Blodgett (2) has made attempts to transmit apple mosaic by insects, mainly aphids and leaf hoppers. Although these attempts were unsuccessful he established that the disease could spread in the orchard and that the spread was chiefly along rather than across the rows. He suggests sap transmission on pruning implements as a possible mode of propagating; this seems unlikely, however, as fruit tree viruses are, in general, non-sap-transmissible.

Clonal Rootstocks as carriers of rubbery wood.

It has already been noted that a number of apple varieties are symptomless carriers of rubbery wood and chat fruit which are transmitted to healthy scions of Lambourne grafted upon them. There seemed a distinct possibility therefore that certain clonal apple rootstocks might also prove to be carriers of these diseases. It was to investigate this possibility that an experiment was started at Long Ashton in 1945 using a selection of the most important East Malling clonal stocks taken from the Long Ashton stoolbeds. These stocks were lined out in rows in the nursery and, in July 1945, were budded with Lord Lambourne material taken from the "original" trees. Nine different sub-clones of Lambourne material were used, each from a different parent tree. All the parent trees were healthy when the bud-wood was collected and none of them have since developed any symptoms of rubbery wood or chat fruit. It seems therefore that there is no possible doubt as to the health of the budding material used in this experiment. Examined at the end of their first year of growth the young trees were found to have made good growth and all were apparently healthy. In November 1947 however, after two years' growth, it became obvious that a large proportion of the trees were in fact rubbery to a greater or lesser degree. A careful survey of these two-year old trees was made by two observers, each tree being classified as healthy, definitely rubbery or doubtful. Although the records were made independently, it will be seen from Table IV that, with the exception of Type VII, there was a large measure of agreement as to the proportion of trees on each stock which were definitely rubbery.

It will also be seen that the different stocks showed well-marked differences in performance. Type II, in particular, was outstanding in giving an almost completely healthy stand of trees. None of the trees on this stock developed definite symptoms of rubbery wood and only a very small proportion were placed in the "suspected" category. The trees were in every way first-class nursery stock. In marked contrast were the trees worked on Type I, of which less than 10% could be said to be really healthy, and 63% of which were very definitely rubbery. The remaining stocks all gave about 20% of definitely rubbery trees with a further 20—30% of doubtfuls.

The different sub-clones of parent Lambourne material, on the other

hand, all showed a relatively uniform performance and all produced about the same proportion of rubbery and healthy trees, the degree of infection recorded depending on the stock on which they were worked. A selection of these trees has been planted out at Long Ashton for further observation.

These results suggest strongly that all the stock types used in this experiment, with the exception of M.II, were carrying the virus of rubbery wood. The fact that most stocks gave a mixture of healthy and rubbery trees, however, shows that the infection was confined to a certain proportion (probably less than 50%) of the stools from which the stocks were taken, and that the whole clone was not infected. As this particular stoolbed is now no longer in existence it has not been possible to investigate this point further, or to suggest how such a large proportion of the stools became infected. The complete freedom from disease of the Type II stocks, however, would seem to indicate that the infection was already present in the other stocks when the stoolbed was originally planted; if the virus had spread after planting one would have expected a more uniform distribution of infected stocks, as there is no evidence to suggest that Type II is immune to (as opposed to a carrier of) rubbery wood.

It is not yet known whether any of the stocks used in this experiment

TABLE IV.

CONDITION OF TWO-YEAR-OLD TREES OF LORD LAMBOURNE BUDDED ON MALLING CLONAL STOCKS, AS RECORDED BY TWO INDEPENDENT OBSERVERS.

Stock	Condition of tree	Sub-clone				Total	Mean %
		1	3	4	5		
I	Total	22	23	21	22	88	
	Healthy	0	1	4	1	6	} 8.5
		3	0	4	2	9	
	Definitely rubbery ..	18	13	7	17	55	} 63.1
		14	18	6	18	56	
	Suspected rubbery	4	9	10	4	27	} 28.4
		5	5	11	2	23	
II	Total	21	20	18	18	77	
	Healthy	20	18	16	16	70	} 91.6
		18	20	16	17	71	
	Definitely rubbery ..	0	0	0	0	0	} 0
		0	0	0	0	0	
	Suspected rubbery ..	1	2	2	2	7	} 8.4
		3	0	2	1	6	
IX	Total	19	22	22	23	86	
	Healthy	7	14	14	11	46	} 45.9
		9	3	9	12	33	
	Definitely rubbery ..	5	5	5	7	22	} 26.2
		5	7	6	5	23	
	Suspected rubbery ..	7	3	3	5	18	} 27.9
		5	12	7	6	30	

TABLE IV—(contd.).

Stock	Condition of tree	Sub-clone				Total	Mean %
		6	7	8	9		
IV	Total	9	17	9	14	49	
	Healthy	6 6	9 9	6 8	5 8	26 31	} 58.2
	Definitely rubbery ..	1 0	3 3	2 1	4 4	10 8	} 18.3
	Suspected rubbery ..	2 3	5 5	1 0	5 2	13 10	} 23.5
VII	Total	36	37	36	36	145	
	Healthy	25 13	24 9	23 12	18 7	90 41	} 45.2
	Definitely rubbery ..	1 6	5 12	3 16	8 20	17 54	} 24.5
	Suspected rubbery	10 17	8 16	10 8	10 9	38 50	} 30.3
XII	Total	9	6	11	9	35	
	Healthy	6 4	4 3	6 8	7 7	23 22	} 64.3
	Definitely rubbery ..	1 5	1 2	2 0	1 1	5 8	} 18.6
	Suspected rubbery ..	2 0	1 1	3 3	1 1	7 5	} 17.1
XVI	Total	22	15	20	12	69	
	Healthy	12 10	6 3	14 6	9 7	41 26	} 48.6
	Definitely rubbery ..	3 4	8 4	3 4	1 2	15 14	} 21.0
	Suspected rubbery ..	7 8	1 8	3 10	2 3	13 29	} 30.4

also carried chat fruit. A selection of trees on each stock has, however, been planted out for observations on fruit size and quality.

Neither is it yet possible to recommend Malling II with confidence as a "safe" stock on which to work Lord Lambourne. Although the particular selection of M.II used in this experiment appeared to be free of rubbery wood it is possible that in other stoolbeds this stock too may be infected. Further indexing trials with Malling stocks from stoolbeds in different parts of the country have recently been started and it is hoped that these will eventually provide further information on this question.

Headworking infected Lord Lambourne trees.

Growers with plantations of Lord Lambourne trees, which, through infection with rubbery wood, chat fruit, or mosaic, have become unprofitable, are faced with the alternatives of grubbing the trees or of headworking or

frameworking them to another variety. For trees severely infected with mosaic there is no doubt that the only sound policy is to grub, and in view of the evidence for the spread of this disease in the field, the earlier this is done the better.

For trees infected with rubbery wood or chat fruit there appears to be no obvious objection to working them over to a non-susceptible variety, and experience has shown that this can be done successfully. Laxton's Superb, in particular, has been observed to make excellent growth and to produce good quality fruit when top-worked on rubbery and chat-fruited Lambourne. Other varieties which have made good growth in the first two years after working on rubbery Lambourne include Crawley Beauty, Blenheim Orange, Edward VII and Monarch. Results with Cox's Orange Pippin have been disappointing: the reason for this is not yet known with certainty, but pending further investigations growers would be well advised to avoid top-working this variety on Lord Lambourne.

DISCUSSION.

The work during the past five years has increased considerably our knowledge of mosaic and rubbery wood in apples and has cleared up a number of points which were formerly in doubt. Owing to the much longer time required for symptoms to show, our knowledge of chat fruit is not yet so far advanced and it is hoped to present a further report on this at a future date.

It has been shown that rubbery wood, like apple mosaic, has all the properties usually associated with virus diseases: it can be transmitted from diseased to healthy trees by grafting; it can spread and multiply within trees of both immune and susceptible varieties; and it appears to have a natural mode of spread in the field.

The sudden appearance of rubbery wood on Lord Lambourne has given rise to some speculation on the origins of the disease. The evidence presented above would seem to suggest that the virus was widely distributed in England long before Lord Lambourne was introduced, but, as most apple varieties are highly tolerant, the virus was noticed only when a susceptible variety was introduced and became diseased. This would explain the occurrence of the disease when healthy Lord Lambourne scions were worked on to apparently healthy stocks. It has, however, also been suggested (7) that in grafting two varieties which are, in themselves, healthy, a protein which is a normal constituent of the cytoplasm of one may, when introduced into the second, become a virus, producing, *de novo*, the disease. If this explanation is accepted, it is difficult to account for the fact that in some cases only a part of a clone appears to be able to transmit the disease, as has been the experience in the trial with Mallings stocks. In any event it must be recognised that, irrespective of origin, a virus disease which has been introduced into a susceptible variety is transmissible and therefore a menace to that variety. For this reason precautions must be taken against its spread and these precautions must take into consideration the possible occurrence of symptomless carriers of the disease.

SUMMARY.

1. This report deals with observations made during the past five years on rubbery wood, mosaic and chat fruit in apples, with particular reference to the variety Lord Lambourne.
2. The symptoms and host ranges of the diseases are described.

PLATE VII



Fig. 1a



Fig. 1b



Fig. 2

Fig. 1a. Healthy tree of Lord Lambourne, headworked on Ontario. Note upright and rather spreading growth habit.

Fig. 1b. Rubbery tree of Lord Lambourne, headworked on Ontario, showing typical drooping habit.

Fig. 2. Young Bramley's Seedling tree headworked with Lord Lambourne in 1945. Rubbery scions were worked on branch 1 and healthy scions on branches 2-5. Note the increasing amount of growth made by the scions, as their distance from the original point of infection increases. On branch 2 much of 2- and 3-year old wood is extremely rubbery; on branches 3-5 marked rubbery symptoms are shown by a few 2-year old twigs. B = a healthy Bramley shoot growing from branch 5.



Plum :—Variety Laxton's Bountiful on Myrobolan B stock, Planted 1938 ; photographed 1948.
A. Healthy. B. Stunted.

3. Rubbery wood and chat fruit were first noticed in the early 1930's, following the headworking of healthy Lord Lambourne on to apparently healthy trees of other varieties. The diseases seem to have appeared spontaneously and independently in different localities at about the same date.
4. Rubbery wood, like mosaic, can be transmitted by grafting and is systemic in trees of both immune and susceptible varieties. It is therefore a virus disease. Chat fruit, too, is probably transmissible by grafting, but the evidence is not yet as conclusive as for rubbery wood or mosaic.
5. Rubbery wood and mosaic, and probably also chat fruit, have a natural method of spread in the orchard.
6. Healthy Lord Lambourne budded on Malling clonal rootstocks showed a large percentage of rubbery trees two years after budding. Only M.II gave a completely healthy stand of trees.
7. Certain varieties of apple, tolerant to rubbery wood and chat fruit, can be successfully headworked on to infected Lord Lambourne trees. Trees infected with mosaic should be destroyed.
8. The above results are discussed in relation to current views on the nature of virus diseases.

ACKNOWLEDGMENTS.

We wish to record our grateful thanks to the following persons without whose assistance the investigations could not have been carried out:— Christopher Norbury, Esq., Sherridge, nr. Malvern; George Nott, Esq., Newnham Farms, nr. Tenbury Wells; Norman McKechnie, Esq., Fairfield's Fruit Farm, Redmarley, Glos.; Andrew Haggard, Esq., Hilltop Fruit Farm, nr. Ledbury.

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VIRUS DISEASES OF FRUIT TREES

III. A PRELIMINARY NOTE ON STUNT OF PLUMS

By S. H. CROWDY and L. C. LUCKWILL.

During the past two years a number of plum trees showing an abnormal habit of growth have been noticed in widely scattered orchards. The appearance of the trees suggested the name "stunt" and the effect appeared to be sufficiently serious to justify further investigation into the cause of the trouble. Plate VIII illustrates the difference between a severely stunted tree and a healthy tree of the same variety and of the same age. Both trees were worked on Myrobolan B and were growing adjacent to each other in the same orchard, and there was no reason to suspect that the trouble was nutritional.

Severely stunted trees are small, the development of the top is poor, and the branches and twigs lack rigidity, giving the crown a flat umbrella shape; there is little extension growth of the branches. The individual shoots are short, flexible and, at the same time, brittle, snapping easily if they are bent too far. Branches of infected trees are usually thinner than branches of corresponding age on a healthy tree. The individual shoots are short, with contracted internodes, and the leaf-bases are enlarged and prominent; the surface of the young shoots is covered with small powdery brown pustules similar to those found in tan bark of cherry. The leaves are of normal size and colour, but are produced rather later in the season than on healthy trees of the same variety. In contrast, flowering is early and profuse. The fruits are normal but mature slightly earlier on infected than on healthy trees. The total crop is much reduced owing to the very small size of the trees. In addition to these external symptoms there is a marked change in the distribution of the tissues of the stem and roots. The wood is very poorly developed and has a wavy outline in transverse section: in contrast, the tissues outside the wood are exceptionally thick and spongy.

Seedling peaches and the cherry root-stock F.12/1 budded with plums of the varieties Bountiful and Giant Prune showing stunt developed characteristic symptoms during the season following budding. The growth of the peach seedlings was markedly reduced, and side shoots were very poorly developed, especially early in the season. The leaves were small and showed faint chlorotic patterns. Later in the season the trees recovered to some extent but remained smaller than the unbudded controls. On cherry the effect on growth was less marked, but the leaves produced early in the season were rather small and had a characteristic mosaic pattern; later formed leaves appeared healthy. No symptoms were observed on infected plum during the season following inoculation, but it seems possible that symptoms of this type in the wood may take more than one season to develop; this has been the experience with rubbery wood in apples (1).

Although the investigations are as yet incomplete the evidence outlined above indicates that a virus infection has been transmitted from the diseased plums, and the stunting effect observed on peach suggests that the virus is the cause of stunting in plums.

Stunt symptoms have been observed on the following varieties: Laxton's Bountiful, Giant Prune, Evesham Wonder and Thames Cross, and possibly

also on Yellow Pershore and Victoria. Affected trees have been seen in the West Midlands, South Eastern and South Western counties.

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EXPERIMENTS ON THE CONTROL OF BROWN ROT OF FRUITS: PROGRESS REPORT 1948-49.

By R. J. W. BYRDE.

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SUMMARY.

In laboratory spore germination tests of seven fungicides, the provisional mean ED-50 values (in $\mu\text{g}/\text{cm}^2$) against *Sclerotinia fructigena* were found to be as follows: phenyl mercury chloride 0.0074; Phygon "XL" 0.03; 8-hydroxyquinoline sulphate 0.058; copper 8-quinolinolate 0.074; Bordeaux mixture (as Cu) 0.21; Experimental Fungicide 341-C 0.33; Lime-sulphur 1.05.

Brown rot infection of plums of the current season was considerably reduced by a late June spray with each of the following materials in a field trial: phenyl mercury chloride 0.0035%, Phygon "XL" 0.1%, copper 8-quinolinolate 0.1%.

Experiments with eradicant fungicides against detached mummified plums infected with *Sclerotinia fructigena* confirmed the effective inhibition of spore formation by phenyl mercury chloride and sodium and calcium arsenite preparations. Sodium dinitro-*ortho*-cresolate and sodium pentachlorophenate also showed some promise as possible winter treatments.

INTRODUCTION.

A previous paper (Byrde, 1948) has described certain preliminary trials in the control of brown rot of apple and plum, caused by *Sclerotinia fructigena* (Ader. and Ruhl.) and *Sclerotinia laxa* (Ader. and Ruhl.). Special reference was made to the use of phenyl mercury chloride and calcium arsenite sprays in winter, which largely inhibited sporulation of mummified fruits in the following summer. An account was also given of the control of the disease on plums in the current season by means of a summer application of a phenyl mercury chloride preparation.

Recently, Calavan and Keitt (1948) have published an account of experiments in Wisconsin (U.S.A.) on the control of blossom and spur blight of sour cherry (caused by *S. laxa*) by means of an eradicant copper-lime-arsenite-oil spray applied just before budbreak. It was found that monocalcium arsenite alone and Elgetol (sodium dinitro-*ortho*-cresolate) were each less effective than the monocalcium arsenite with copper sulphate, lime and fish oil added. On the other hand, Miller (1949), reporting on 1949 experiments in California, states that the addition of Bordeaux mixture and 0.5% petroleum oil to a 0.3% monocalcium arsenite preparation, applied to apricots in January, did not affect its eradicant action against *S. laxa*.

In view of the serious damage caused to almonds by arsenite sprays, Wilson (1943a; 1943b) attempted to find a less phytotoxic alternative. Sodium pentachlorophenate and sodium tetrachlorophenate were the most

* The work described was carried out at Long Ashton Research Station, by kind permission of the Director, to whom grateful thanks are due, with the aid of a Further Education and Training Grant through the Ministry of Agriculture.

effective compounds tested, but were nevertheless inferior to the arsenites as eradicant fungicides.

EXPERIMENTAL.

I. Laboratory Spore Germination Tests.

A spore germination experiment, using a horizontal sprayer technique, was carried out on seven fungicides which had shown promise in preliminary tests. The fungicides were tested three at a time in a balanced incomplete block design, permitting statistical analysis to eliminate day-to-day variations (Finney 1947; p. 82). Each material was tested three times, and altogether seven series of tests were carried out.

The following fungicides were used:—

(a) *Standard laboratory Bordeaux mixture*, prepared as recommended by the American Phytopathological Society, Committee on standardization of fungicidal tests (1943 b).

(b) *8-Hydroxyquinoline sulphate*, as an aqueous solution of the neutral monohydrate.

(c) *Phygon "XL"*, containing 50% 2:3-dichloro-1:4-naphthoquinone, as an aqueous suspension.

(d) *Phenyl mercury chloride*. A stock suspension, for dilution as required, was prepared by grinding together:

Phenyl mercury chloride	..	..	..	10 gm.
10% Sulphite lye	..	..	..	100 ml.

(e) *Lime-sulphur*, as an aqueous solution.

(f) *Copper 8-quinolinolate*. A stock suspension, for dilution as required, was prepared by grinding together:

Copper 8-quinolinolate	..	..	..	10 gm.
10% sulphite lye	..	..	..	50 ml.

(g) *Experimental Fungicide 341-C*, containing 54% 2-heptadecylglyoxalidine, as an aqueous solution to which was added 0.25 gm. calcium hydroxide per ml. of fungicide concentrate.

The technique used was that recommended by the American Phytopathological Society, Committee on standardization of fungicidal tests (1943 a), using the horizontal sprayer described by Evans and Martin (1935).

Spraying was carried out at a pressure of two atmospheres, and the glass slides, previously coated with cellulose nitrate, were held at a distance of two feet from the spray nozzle. This was adjusted to give a relatively low output, and deposition was found by chemical analysis to be at the rate of 41.67 μ gm. of spray per sq. cm. of slide per second. By varying the time of spraying from 5 to 80 seconds, a series of nine deposits was obtained, with a dose ratio of $\sqrt{2}$. The relative humidity of the atmosphere in the laboratory at the time of spraying varied between 58% and 70% on the seven occasions.

Spores were obtained from seven-day old cultures of *S. fructigena* on autoclaved potato plugs previously soaked in 7½% malic acid for 72 hours. The cultures were kept in the incubator for four days at 28° C., then exposed to light under laboratory conditions for a further three days, to encourage sporulation. Spores were removed by washing and centrifuging, and a suspension prepared at a concentration of 50,000 spores /ml. in 0.1% Orange Juice. Drops of approximately 0.05 ml. of this suspension were applied by means of a standard 1 ml. pipette to the deposits on the glass slides after these had been allowed to dry in a dustproof place for 5 to 6 hours. The

drops spread to a diameter of approximately 7.5 mm., except in the case of the heavier deposits of Experimental Fungicide 341-C, where lowered surface tension caused some increase of spread.

Slides were incubated at $28^{\circ} \pm 1^{\circ}$ C. for 20 hours. The germination of 100 spores was recorded for each fungicidal treatment; Control and "Prior" Germination were estimated from a count of 200 spores each.

Table I shows the mean ED-50 values (averaged logarithmically) obtained for each fungicide, as estimated from the three provisional probit lines obtained for each. Results are being computed from the lines mathematically and a statistical analysis of the calculated ED-50 values is being carried out and will be published elsewhere.

TABLE I.
PROVISIONAL MEAN ED-50 VALUES AGAINST *S. fructigena*.

Fungicide					Mean ED-50 ($\mu\text{gm./cm}^2$)
Phenyl mercury chloride	..	..	..	..	0.0074
Phygon "XL"	..	..	..	..	0.030
8-Hydroxyquinoline sulphate	..	..	..	..	0.058
Copper 8-quinolinolate	..	..	..	..	0.074
Standard laboratory Bordeaux mixture (expressed as Cu)	..				0.21
Experimental Fungicide 341-C	..	..	..	..	0.33
Lime-sulphur	..	..	..	..	1.05

Using this basis of comparison, the relative fungicidal values of these materials, taking lime-sulphur as 1.0, could be expressed as follows: Experimental Fungicide 341-C 3.2; Bordeaux mixture (as Cu) 5.0; Copper 8-quinolinolate 14.2; 8-hydroxyquinoline sulphate 18.1; Phygon "XL" 35.0; phenyl mercury chloride 142.

II. Summer Spraying of Plums.

The objects of this experiment were to investigate the field performance, as summer sprays, of fungicides showing greatest promise in the laboratory spore-germination tests, and, at the same time, to confirm the satisfactory results from using phenyl mercury chloride as a late June spray in 1948.

The trial was carried out on sixteen 15-year old standard Victoria plum trees, planted at approximately 24 feet apart in a single row. The following four treatments were each applied to four trees, arranged in blocks to minimise spread of the disease between treatments:

- | | | | | |
|-----|--|----|----|---------|
| (a) | Phenyl mercury chloride | .. | .. | 0.0035% |
| | Kaolin | .. | .. | 0.14% |
| | Estol "H" | .. | .. | 0.25% |
| (b) | Phygon "XL" (containing 50% 2:3-dichloro-1:4 naphthoquinone) | .. | | 0.1% |
| | Estol "H" | .. | .. | 0.125% |

Estol "H" was used at half the normal rate, as the Phygon "XL" is itself a wettable powder.

- | | | | | | |
|-----|------------------------|----|----|----|-------|
| (c) | Copper 8-quinolinolate | .. | .. | .. | 0.1% |
| | Estol "H" | .. | .. | .. | 0.25% |
| (d) | Control—no spray | .. | .. | .. | |

One day before spraying, four mummified plums bearing spring pustules of *S. fructigena* were hung in each tree on separate branches, in order to provide a fairly uniform source of infection. No other mummified fruits were present on the trees.

The fungicides were applied on 23rd June, 1949, by means of a bucket sprayer, each tree receiving approximately 4 gallons of spray. Care was taken to ensure thorough wetting of the fruits. Weather conditions were dry and warm: no rain fell during the ensuing eleven days.

On August 16th–17th, 1949, 200 fruits were picked from each of the sixteen trees in this trial, taking them from a convenient cone-shaped section of each tree, extending to the centre. Fruit showing any brown rot infection was classified as infected. Table II summarises the results obtained.

TABLE II.
BROWN ROT INFECTION OF VICTORIA PLUM ON 16TH–17TH AUGUST, 1949.

Treatment	No. of infected fruit out of 800 picked	% Infection
Phygon "XL" 0.1%	1	0.12
Phenyl mercury chloride 0.0035% ..	3	0.37
Copper 8-quinolinolate 0.1%	7	0.87
Control ..	26	3.25

As in 1948, there was considerable heterogeneity within treatments, but a Chi-square test again indicates significant differences between treatments as a whole. Further comparison between pairs of treatments suggests that each fungicide is a significantly better treatment than Control, but does not show a significant difference between any pair of fungicides.

It will be noted that the brown rot infection was slight even on Control trees; in the 1948 trial, the comparative figure for Control was 22%.

At leaf-fall in November, 1949, it was observed that the leaves of sprayed trees remained free from rust (*Puccinia Pruni-spinosae* (Pers.)) and were retained two to three weeks longer than those of the Control trees, which were severely rusted.

Phytotoxic effects

Preliminary trials indicated that phenyl mercury chloride was liable to damage fruit of Victoria plums at the usual summer concentration of 0.005%, which, in a slightly different preparation, proved non-toxic to Belle de Louvain plums in 1948. The material was therefore used at 0.0035%. Even at this concentration slight brown necrotic spotting was observed at the base of

each fruit, due to accumulation of spray by drainage : damage was confined to this area.

Considerable damage to the fruits was caused by Phygon "XL," in the form of dark brown necrotic spotting over the entire fruit. This was still noticeable at picking time, and many of the fruits had not developed to a normal size.

No damage resulted from the copper 8-quinolinolate spray.

III. Experiments with Eradicant Fungicides on Detached Mummified Fruits.

In order to investigate further the relative efficiency of various eradicator sprays against brown rot, six such materials were tested, each at five concentrations, with a dose ratio of 2, against detached mummified plums infected with *S. fructigena*. These fruits had previously received a routine spray in the orchard of 0.1% D.N.C. in 3% petroleum oil. Thirty fruits were allotted per treatment.

The fungicides tested were :

(a) *Phenyl mercury chloride*. A stock suspension, for dilution as required, was prepared by grinding together :

Phenyl mercury chloride	..	..	..	10 gm.
10% Sulphite lye	..	..	..	100 ml.

The material was used at concentrations ranging from 0.4% to 0.025%.

(b) *Sodium dinitro-ortho-cresolate*. A 60% preparation of the above salt was used as an aqueous solution. Concentrations are expressed as percentages of the pure material, and ranged from 0.8% to 0.05%.

(c) *Sodium pentachlorophenate*, as an aqueous solution, at a range of concentrations from 1.6% to 0.1%.

(d) *Sodium arsenite*, as an aqueous solution.

(e) *Monocalcium arsenite*, as an aqueous suspension prepared from calcium hydroxide and arsenious oxide, as described by Byrde (1948).

(f) *Monocalcium arsenite plus Bordeaux mixture*. A monocalcium arsenite suspension was added to diluted Bordeaux mixture in the proportion of 2 parts arsenite—3 parts $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ —2 parts $\text{Ca}(\text{OH})_2$.

The three arsenical preparations were each used at concentrations ranging from 0.4% to 0.025%.

To ensure adequate wetting of fruits, Estol "H" at 0.25% was added to each concentration of every fungicide.

On 28th–29th April, 1949, fruits were dipped for five seconds each in the appropriate fungicide in a beaker. They were placed immediately on cellulose-sprayed wire-netting trays and protected from rain for twenty-four hours before being placed on a rack structure in the open. 30 fruits were left untreated as Controls.

On 27th July, 1949, an estimate was made of the number of pustules of unit size on each fruit, and the mean number of such pustules per fruit determined for each treatment. The graphs constructed (*Figures 1 and 2*) show the inhibition of pustule production relative to the untreated fruits, plotted as probits against the logarithm of the concentration in p.p.m.

The mean number of pustules per fruit from Controls was 118.0.

From the graphs an estimate may be obtained of the approximate ED-95 value for each fungicide. These estimates are shown in Table III.

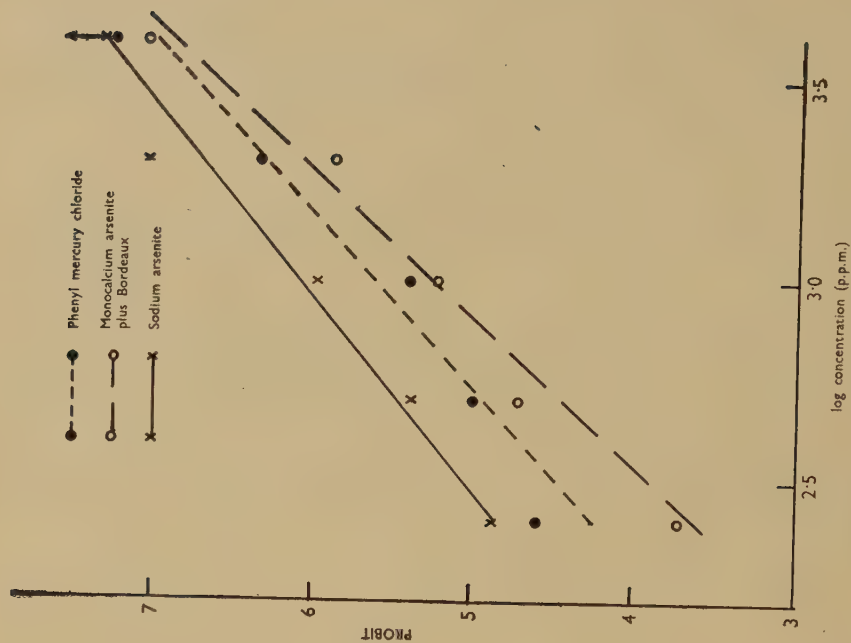


Fig. 2. Inhibition of pustule production of *S. fructigena* by eradicant fungicides.

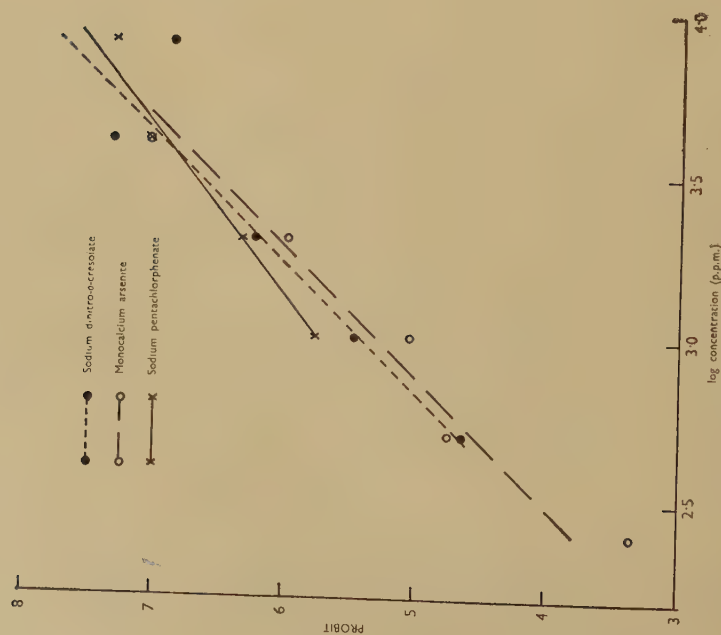


Fig. 1. Inhibition of pustule production of *S. fructigena* by eradicant fungicides.

TABLE III.

APPROXIMATE ED-95 VALUES OF VARIOUS FUNGICIDES AS INHIBITORS OF SPORULATION OF DETACHED MUMMIFIED PLUMS.

Fungicide	Approximate ED-95 (%)
Sodium arsenite	0.18
Sodium pentachlorophenate	0.28
Phenyl mercury chloride	0.28
Sodium dinitro-ortho-cresolate	0.30
Monocalcium arsenite	0.32
Monocalcium arsenite plus Bordeaux mixture ..	0.32

These results show that sodium arsenite was the most effective of the materials tested, all of which, however, when used at 0.3%, would inhibit sporulation by approximately 95%.

Effect of oils on eradicant properties of phenyl mercury chloride.

A comparison between various oils incorporated with phenyl mercury chloride sprays was made on 260 mummified plums not previously treated with any spray material. Phenyl mercury chloride was used at three levels of concentration, each with four oil emulsion treatments:

JD-2 petroleum oil 3%

Tar-oil 3%

JD-2 petroleum oil 3% with 0.1% D.N.C.

No oil.

Oil emulsions were prepared with sulphite lye, and added to the diluted phenyl mercury chloride suspension. Estol "H," at 0.25%, was used throughout as wetting agent.

Twenty fruits were used per treatment, with 20 untreated fruits as Controls.

Fruits were dipped, as described above, on 25th March, 1949, and recordings carried out on 23rd July, 1949. Table IV shows the mean number of pustules of unit size per fruit for the various treatments; the comparable figure for Controls was 140.8.

TABLE IV.

MEAN NO. OF PUSTULES PER FRUIT FROM BATCHES OF 20 MUMMIFIED PLUMS TREATED WITH PREPARATIONS OF OILS AND PHENYL MERCURY CHLORIDE.

PMC concn. %	JD-2	Tar-Oil	JD-2 & DNC	None
0.2	14.1	8.15	13.3	20.0
0.1	48.25	45.5	60.25	72.0
0.05	125.5	62.35	102.5	74.0

Analysis of variance of these data shows that there is no significant difference between oil treatments but that concentration differences are

significant. The least difference between means of concentrations required for significance ($P = 0.05$) is 31.2.

DISCUSSION

Laboratory spore germination tests have again demonstrated the very high toxicity of phenyl mercury chloride to spores of the brown rot fungi. When considering these results with a view to performance in the field, however, it is necessary to bear in mind that phenyl mercury chloride is used at a low concentration—of the order of 0.005%—in summer sprays, compared with a concentration of 0.1% for most of the other materials tested in the laboratory. The reverse is true in the case of lime-sulphur, which is generally applied at 1%. On the basis of these laboratory tests, phenyl mercury chloride, Phygon "XL" and copper 8-quinolinolate were chosen for the field trial on plums.

Despite the lightness of the brown rot attack on the Victoria plums used in the 1949 trial, it is encouraging that the results for the phenyl mercury chloride treatment bear out those of the more normal 1948 season. A good control of the disease was again obtained, but the material suffers from two disadvantages—its phytotoxicity under certain conditions, and the fact that, owing to its poisonous nature, it cannot be applied late in the season.

Phygon "XL," although providing excellent disease control, caused considerable damage at 0.1%, and preliminary experiments not described in this paper showed that slight damage occurred at as low a concentration as 0.03% on Victoria plums. This material, as at present formulated, therefore appears to be of little promise for general use.

Copper 8-quinolinolate, at 0.1%, caused no damage and gave a satisfactory control of the disease. On the other hand, it is considerably more expensive than the mercurial at the concentrations used. Further tests will be carried out with this material and with phenyl mercury chloride as summer sprays on plums, but it is too early to make any recommendations for their more general use.

The results of field experiments in the use of phenyl mercury chloride as an eradicant winter spray are being held over until they can be presented with those obtained in a more normal season. The tests on detached mummified plums described in this paper indicate the approximate concentration of eradicant fungicides required to obtain any given reduction in sporulation. It will be seen that phenyl mercury chloride has to be used at about 0.3% to obtain an inhibition of 95%, which confirms the findings of 1947-48 experiments. A spray of such a concentration—about sixty times that of the preparation used for summer spraying—is very costly, however. Sodium pentachlorophenate and sodium dinitro-*ortho*-cresolate show some promise as alternatives, but in considering the results obtained it should be noted that these materials were applied to the mummified fruits in dry weather in late April. Field spraying would have to be done in the dormant season and there is a considerable likelihood of the soluble sodium salts being washed out quickly, with consequent loss of fungicidal efficiency.

In a second test it was not possible to show any significant effect resulting from the incorporation of oils with phenyl mercury chloride sprays.

Monocalcium and sodium arsenites again proved to be efficient eradicant fungicides. It is interesting, in connection with the conflicting claims from the U.S.A., to which reference has already been made, that there appears to be no difference in eradicant action between monocalcium arsenite alone,

and the material with Bordeaux mixture added ; in fact, the two graphs presented are virtually concurrent.

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EXPERIMENTS WITH SYSTEMIC INSECTICIDES AGAINST THE GREEN APPLE APHIS—*APHIS POMI*

By S. H. BENNETT.

The study of systemic insecticides and their practical use has progressed rapidly since the original work on the phosphorus compounds by Schrader, Kükenthal and their colleagues in Germany (1 & 2). In the 1947 report (3) the systemic insecticidal properties of bis(β -fluoroethoxy)methane against *Aphis fabae* and the larvae of *Callimorpha jacobaeae*, *Pionea forficalis*, *Pieris brassicae* and *rapae*, *Plutella maculipennis* and *Mamestra brassicae* were described. In 1949 (4) the results were given of experiments with bis(β -fluoroethoxy)methane, bis(dimethylamino)fluorophosphine oxide and bis(dimethylamino)phosphonous anhydride. The fumigant action of the bis(dimethylamino)fluorophosphine oxide from aqueous solution, and from the leaves of plants which had absorbed the material through the roots, was demonstrated on adult Brassy Willow Beetles, *Phyllodecta vitellinae*. The fumigant action of the materials on larvae of *Pieris brassicae* and *Diataraxia oleracea* has also been described (5). In these studies herbaceous plants were used. Other experiments have now been extended to perennial woody plants, special attention being paid to the residual insecticidal toxicity of the materials. This is a point of extreme importance in view of the high mammalian toxicity of the materials, and one which must be carefully studied before field uses are considered.

TECHNIQUES AND MATERIALS USED.

The plants used in the experiments were apple seedlings, raised in 4" pots in an unheated greenhouse. By the end of April they were 4"-5" high and had on an average seven open leaves. Some plants were repotted into 6" pots at the beginning of June when they were 12"-15" high. Apple seedlings grown in this way are very useful test plants for this type of work. The growth is straight, the leaves are well spaced, enabling easy treatment of individual leaves if necessary, yet the total spread of leaves from the main stem is not great, which facilitates the collection of the dead aphides as they fall from the plant.

The method of infesting plants was to collect either foliage or blossom trusses which carried a natural infestation of the Green Apple Aphis. Such trusses each bore upwards of 100 aphides both large and small. Immediately after collection a single truss was placed in the axil of a leaf about halfway up the main stem of the test plant, allowing plenty of contact between the truss and the plant. As the truss wilted the aphides became restless and wandered on to the test plant. In nearly all cases, all the aphides had migrated from the wilting truss within 24 hours, after which time the truss was removed.

To collect the dead aphides that fell from the plant, stiff white paper circles were cut, large enough to more than cover the leaf span of the plant. A cut was made from the edge to the centre and here a triangular cut was made so that the paper fitted round the main stem. The edges of the paper were greased with a $\frac{1}{2}$ " band of tree banding grease to trap any aphides wandering off should the treatment prove repellent. To support the paper, and to keep it flat, cardboard circles cut in a similar way were used. The dead aphides falling from the plant could be counted and removed daily from the

paper, but it was found impracticable to remove all dead aphides from the plants daily. In many cases the aphides died with their stylets still inserted in the plant tissue and as their removal might have interfered with live insects on the plant they were not recorded until the end of the experiment, when all aphides, whether alive or dead, were brushed from the plant and counted.

SOIL TREATMENTS.

Aqueous solutions of the following materials were applied to the soil of pots in which apple seedlings were growing.*

- (1) bis(β -fluoroethoxy)methane, $\text{CH}_2(\text{OCH}_2\text{CH}_2\text{F})_2$ (F)
- (2) bis(dimethylamino)fluorophosphine oxide, $[(\text{CH}_3)_2\text{N}]_2\text{POF}$ (D)
- (3) bis(dimethylamino)phosphonous anhydride,
 $[(\text{CH}_3)_2\text{N}]_2\text{PO.O.PO} [\text{N}(\text{CH}_3)_2]_2$ (P)
- (4) bis(dimethylamino)-8-hydroxyquinolyl-phosphine oxide,
 $[(\text{CH}_3)_2\text{N}]_2\text{PO.O.C}_9\text{H}_6\text{N}$ (Q)
- (5) Sodium selenate, Na_2SeO_4
- (6) "Pestox 3" a commercial preparation estimated to contain 75%—
 80% bis(dimethylamino)phosphonous anhydride,
 $[(\text{CH}_3)_2\text{N}]_2\text{PO.O.PO} [\text{N}(\text{CH}_3)_2]_2$ (Pestox)

All materials were applied at the rates of 50, 125, 250 and 500 p.p.m. of the average weight of moist soil in 4" pots, except P which was applied at the rate of 500 p.p.m. only. In all cases the insecticides were watered onto the soil in the same volume of water and no precautions were taken to prevent leaching of the materials by normal overhead watering.

The plants were infested with *A. pomi* on 13.4.49 so that infestations were well established on all plants when the insecticides were applied on 21.4.49. Daily counts of the fallen dead aphides were made 22.4.49 to 3.5.49, after which time all aphides, whether alive or dead, were removed from the plants.

Subsequent infestations were made 12, 21 and 42 days after treatment and daily counts were made for a period of 7 days following each infestation.

The results of the four infestations are given in Table I.

Phytotoxicity.

D proved extremely phytotoxic, the first symptoms appearing as a marginal scorch four days after treatment and the damage increasing with the concentration. The plants treated at 500 and 250 p.p.m. were so badly damaged that only one infestation was possible. The damage on the plants treated at 50 p.p.m. closely resembled potash deficiency, but on analysis of the leaf tissues no difference in potash status was found between them and a normal untreated plant.

Plants treated with sodium selenate at 500 and 250 p.p.m. showed scorch spots within 5 days, and after 10 days the leaves were brittle though retaining a green coloration. Small necrotic spots appeared on the leaves at 125 p.p.m.

P at 500 p.p.m. produced marginal scorch on the older leaves, but younger leaves developing later showed no scorch.

Q at 500 p.p.m. produced very slight marginal damage on the older leaves, and Pestox at 500 p.p.m. a very slight tip scorch. F at 500 p.p.m. killed the growing point but at 250 p.p.m. side shooting took place and the growth was slow but normal.

* For the remainder of the paper, the materials will be referred to by the code letter following the formula.

TABLE I.
PERCENTAGE KILLS OF *A. pomi* ON APPLE SEEDLINGS BY RESIDUAL SYSTEMIC
TOXICITY AFTER SOIL APPLICATION.

Infestation made Days after Treatment	Material	Amounts applied to the soil in p.p.m.							
		500 p.p.m.		250 p.p.m.		125 p.p.m.		50 p.p.m.	
		% Kill	After Time in Days	% Kill	After Time in Days	% Kill	After Time in Days	% Kill	After Time in Days
0	Pestox	100	7	100	7	100	7	100	7
	P.	100	3						
	D.	100	2	100	2	100	2	100	2
	F.	100	3	100	3	100	3	100	3
	Q.	100	9	100	9	100	10	65	12
	Na ₂ SeO ₄	100	8	100	11	85	12	65	12
	Control	71	12						
12	Pestox	100	1	100	3	100	2	100	3
	P.	100	1						
	D.					100	1		
	F.	100	1	100	1	100	1	100	3
	Q.	9	7	28	7	39	7	9	7
	Na ₂ SeO ₄					100	7	48	7
	Control	16	7						
21	Pestox	100	2	100	2	100	3	100	2
	P.	100	1						
	D.					100	1		
	F.	100	1	100	2	100	2	100	3
	Q.	33	7	58	7	3	7	12	7
	Na ₂ SeO ₄					43	7	94	7
	Control	60	7						
42	Pestox	100	2	100	3	100	3	100	4
	P.	100	1						
	D.								
	F.			100	1	100	3	100	3
	Q.	0	7	0	7	4	7	1	7
	Na ₂ SeO ₄					67	7	50	7
	Control	2	7						

Insecticidal Activity.

In Table I it is possible to show the time after which 100% kill is obtained, or the percentage kill at the end of the experiment. Some points emerging from the more detailed results are worthy of mention. At the first infestation the kill was much quicker on the plants treated with F and D than on any of the other treatments, though subsequent infestations did not show this difference in speed of action to the same extent. This suggests that the quicker kill is due to the fumigant properties which F and D are known to possess, or that the absorption and distribution through the plant is more rapid for F and D than for the other materials.

When assessing the results, the phytotoxicity already noted must be taken into account. For instance, the higher concentrations of F and D gave 100% kill of aphides in the first infestation, before any phytotoxic symptoms were noticeable, but in the case of sodium selenate severe damage was obvious before 100% kill was obtained and the kill may be directly associated with phytotoxic damage and starvation.

The persistence of the materials as insecticides when applied to the soil is clearly shown in Table I. P at the rate of 500 p.p.m. gave 100% kill in 24 hours six weeks after treatment, while Pestox even as low as 50 p.p.m., was

giving 100% kill in 4 days after six weeks. F at 50 p.p.m. was giving 100% kill in 3 days after six weeks. Q at the higher concentrations gave 100% kill in the first infestation but showed no residual toxicity.

After six weeks, the plants became badly attacked by Powdery Mildew, *Podosphaera leucotricha*, and were no longer suitable for detailed experiments. They were kept, however, and infested again on 8.7.49, nearly four months after the application of the materials. As the aphides rapidly became winged and the condition of the plants was unfavourable for aphid infestation no counts were made, but it appeared that plants in pots treated with P or Pestox at 500 p.p.m. or F at 250 p.p.m., were still insecticidal.

LEAF TREATMENTS.

These experiments were designed to give information on the contact effect of the materials as well as on their residual toxicity when absorbed through the leaves. The apple seedlings were growing in 4" pots and at the time of treatment were 4"—5" high and had 8-10 leaves. The technique adopted for dipping was to place a piece of cardboard round the stem of the plant and over the top of the pot. The pot was then inverted and the leaves and stem dipped into the solution and gently agitated to ensure as complete a wetting as possible. The pot was placed on its side to allow any excess solution to drip off and the plant to dry. This procedure prevented contamination of the soil in which the plants were growing.

Contact Action.

Infestations of *Aphis pomi* were allowed to become well established on the plants before dipping. Three concentrations—1.0%, 0.1% and 0.01%—of aqueous solutions of Pestox, P, D, F, Q and sodium selenate were tested, and a wetting agent, "Estol H," was added, at the rate of 1-300 which is slightly higher than that normally recommended for field application. The method of recording the results was the same as that already described for the soil treatments.

The results of two separate experiments made 28.4.49 are given in Table II.

In an experiment of this nature, where the materials are known to have systemic properties, it is difficult to separate contact and systemic action. It has been reported (6) that the direct contact action of Pestox against aphides is very weak and that there is no contact action by residual film. If this is so, then the results in Table II would indicate absorption and translocation in under 24 hours. As a later table will show, however, in only one instance has 100% kill been obtained in 24 hours on residual treatments, where the insecticide must have been in the plant tissue at the time of infestation. This suggests that the contact action of Pestox and P may be quite high. With 1.0% and 0.1% D and F the kill at the end of 24 hours was over 95% and reached 100% in 4-5 days—a time lag which may represent the time taken for the systemic action of these materials to become effective.

Residual Toxicity after Leaf Treatments.

The residual toxicity was assessed by making infestations at intervals after treatment and the results are given in Table III. Na_2SeO_4 at 1.0% proved phytotoxic. It will be seen from these results that the only materials showing persistence of effect when applied to the foliage are P and Pestox. Even 34 days after treatment with 1.0% the plants were toxic to aphides,

TABLE II.

APHIS MORTALITY ON INFESTED PLANTS AFTER DIPPING THE LEAVES IN INSECTICIDES.

Treatment	Concentration of Materials in Aqueous Solution					
	1.0%		0.1%		0.01%	
	% Kill	After time in days	% Kill	After time in Days	% Kill	After time in days
A. Pestox	100	1	100	1	30	8
P.	100	1	100	2	76	8
D.	100	4	100	4	99	8
F.	100	4	100	4	88	8
Q.	99	8	60	8	11	8
Na ₂ SeO ₄	98	8	78	8	26	8
Estol H	18	8				
Control	3	8				
B. Pestox	100	1	100	1	50	5
P.	100	1	100	1	100	3
D.	100	1	100	1	88	5
F.	100	5	100	1	95	5
Q.	96	5	53	5	50	5
Na ₂ SeO ₄	100	2	92	5	36	5
Estol H	22	5				

TABLE III.

PERCENTAGE KILLS OF *Aphis pomi* ON APPLE SEEDLINGS BY RESIDUAL SYSTEMIC TOXICITY AFTER LEAF TREATMENTS.

Infestation made. Days after Treatment	Material	1.0%		0.1%		0.01%	
		% Kill	After time in days	% Kill	After time in days	% Kill	After time in days
4	Pestox	100	2	100	2	100	6
	P.	100	2	100	2	43	8
	D.	100	6	3	8	< 3	8
	F.	3	8	< 3	8	< 3	8
	Q.	< 1	8	< 3	8	< 3	8
	Na ₂ SeO ₄			< 3	8	11	8
	Control	< 3	8				
12	Pestox	100	3	100	3	8	8
	P.	100	2	100	2	11	8
	D.	93	8	< 2	8	4	8
	F.	13	8	10	8	< 2	8
	Q.	< 2	8	< 2	8	< 2	8
	Na ₂ SeO ₄			4	8	< 2	8
	Control	< 2	8				
21	Pestox	100	1	100	3	5	7
	P.	100	2	100	3	12	7
	D.	32	7			31	7
	F.	10	7	< 12	7	25	7
	Q.	29	7	14	7	40	7
	Na ₂ SeO ₄			60	7	13	7
	Control	12	7				
34	Pestox	100	3	5	8	7	8
	P.	100	3	13	8	< 1	8
	D.	< 1	8	< 1	8	< 1	8
	F.	< 1	8	< 1	8	< 1	8
	Q.	< 1	8	< 1	8	< 1	8
	Na ₂ SeO ₄			< 1	8	< 1	8
	Control	< 1	8			< 1	8

giving 100% kill within three days. In this time interval the plants had grown to some two or three times the size they were when treated. No detailed results could be obtained after the 34 day interval but an infestation made 71 days after treatment suggested that plants treated with 1.0% P or Pestox were no longer toxic to aphides.

The non-persistence of D, and particularly F, is interesting, as they persist when applied to the soil. It may mean that leaves are unable to absorb these materials to the same extent as P or Pestox, or it may be associated with their volatility—both materials we know can be given off in the vapour phase from plants. It may also mean that with soil treatment D and F are only slowly absorbed from the soil.

SUMMARY

- (1) Experiments were carried out to discover the contact and residual toxicity of certain systemic insecticides when applied either to the foliage of apple seedlings or to the soil in which these seedlings were growing.
- (2) Of the materials tested by making applications to the soil in which apple seedlings were growing, bis(dimethylamino)phosphonous anhydride, "Pestox 3," bis(β -fluoroethoxy)methane and bis-(dimethylamino)fluorophosphine oxide showed persistent systemic toxicity to *Aphis pomi*. All gave 100% kill within 4 days, 42 days after treatment with amounts as low as 50 p.p.m.
- (3) Soil applications of bis(dimethylamino)fluorophosphine oxide, bis-(β -fluoroethoxy)methane and Na_2SeO_4 were phytotoxic to apple seedlings, bis(dimethylamino)fluorophosphine oxide causing complete death of the plant at 500 p.p.m. and producing a marginal scorch at 50 p.p.m.
- (4) When applied by dipping the leaves in 1% aqueous solutions all the materials gave an almost complete kill of *Aphis pomi* but only bis(dimethylamino)phosphonous anhydride, "Pestox 3," bis(dimethylamino)fluorophosphine oxide and bis(β -fluoroethoxy)methane, gave high kills at lower concentrations.
- (5) After application of aqueous solutions of the materials to the leaves of apple seedlings the greatest persistent systemic toxicity to *Aphis pomi* was shown by bis(dimethylamino)phosphonous anhydride and "Pestox 3." 34 days after application of a 1.0% solution to the leaves, a 100% kill of aphides was obtained in 3 days. Bis(dimethylamino)fluorophosphine oxide at 1.0% showed fairly strong persistence for 12 days but the other materials tested showed no persistence.

ACKNOWLEDGMENTS.

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A SUMMARY OF FRUIT SPRAYING PROGRAMMES: 1949 REVISION.

By H. G. H. KEARNS and R. W. MARSH.

Since the last revision of the spraying programmes in the 1941 Annual Report of the Long Ashton Research Station a number of valuable new plant protectants have become available and more experience in the uses of the older materials has been gained. Reference is made only to new materials which have stood the test of widespread successful use. It is for this reason that no reference is made to "Parathion," as insufficient experience has been gained of its toxicity to man.

The more important advances in knowledge of the treatment of insect and fungus pest of fruit include the control of Apple Blossom Weevil by DDT, the substitution of winter washing on apples and pears by the efficient application of DDT emulsion (not suspensions) at bud burst, the control of Leaf Curling Currant Midge by DDT, and the control of Pear Midge by the application of tar oil washes to the soil.

Further studies on the use of DNC petroleum washes on apples have shown that the control of Apple Sucker and of Red Spider winter eggs obtained by these washes is insufficient. It is therefore necessary to supplement the use of these materials and modify the programme when these pests are present.

The general development of "automatic" spraying introduces new problems in the risk of damage by drift to the crop and in hazards to operators of the machine. It becomes necessary under some conditions to reduce the concentration of the lime sulphur washes, and tentative experiments suggest that, in "automatic" spraying, 1 per cent lime sulphur may be satisfactory at all pre-blossom stages on apples and pears.

On strawberries, complete control of aphids, which is essential for the control of virus, still remains difficult to achieve unless nicotine fumigation under a dragsheet is practised. Other materials such as "Parathion" are highly satisfactory but their application is accompanied by undetermined hazards to health.

For further particulars of individual pests, diseases and materials the reader is referred to the programmes in the 1936 and 1941 Long Ashton Annual Reports.

The spray programmes described cover the following crops :—

I Apple	VII Gooseberry
II Pear	VIII Raspberry
III Plum	IX Loganberry
IV Cherry	X Blackberry
V-VI Black Currant, Red and White Currants	XI Strawberry

The stages of flower bud development used in the programmes are those illustrated in Bulletin No. 137 of the Ministry of Agriculture.

I—APPLE.

Stage	Approx. date of spraying	Materials per 100 gal. wash	Pests and Diseases Controlled
1 Dormant	Dec.-mid March	4-7½ gal. tar oil emulsion	Sucker Aphides
2 Delayed dormant	late March	7½ gal. petroleum oil emulsion	Capsids Winter Moths Red Spider ⁽¹⁾ (some control)
or 2a Delayed dormant (in place of 1 & 2) or 2b Bud burst (aphis egg hatch) (in place of 1 & 2 or of 2a)	late March— early April	7½ gal. DNOC-petroleum oil emulsion DDT emulsion and wetter ⁽⁴⁾	See above ⁽¹⁾ ⁽²⁾ ⁽⁵⁾ Sucker ⁽²⁾ Aphides Apple Blossom Weevil Winter Moths Tortrix Moths Capsids
3 Green cluster	mid April	2 gal. lime sulphur	Scab ⁽³⁾
4 Pink Bud	early May	2 gal. lime sulphur	Scab ⁽³⁾
5 80% petalfall	mid-end May	1 gal. lime sulphur 8oz. nicotine, wetter ⁽⁴⁾	Scab, Sawfly Red Spider Capsids
6 3 weeks after petalfall	mid June	¾ gal. lime sulphur wetter ⁽⁴⁾	Scab Red Spider
7 Codling cover wash	late June— early July	3 lb. lead arsenate powder ⁽⁶⁾	Codling Moth

(1) Washes containing petroleum oil applied late give about 85 per cent control of winter eggs which is insufficient in many seasons and localities.

(2) Control of apple sucker (*P.mali*) insufficient unless trees sprayed with tar oil within the past five years.

(3) If sprayed with "automatic" machines it may be necessary to reduce concentration to 1 per cent on account of drift.

(4) For example, not less than 3 pints of Shellestol or 1 lb. of sulphonated loral or wetter of equal wetting properties and compatible with the wash.

(5) If capsid absent apply lower concentration.

(6) Omit on all early harvested varieties.

Alternative Spray Materials.

Copper Sprays. Bordeaux mixture may be used pre-blossom instead of lime sulphur on varieties not copper-sensitive (see below). A wash may be prepared with 8 lb. copper sulphate (25 per cent copper) and 12 lb. hydrated lime per 100 gallons water. The copper sulphate and hydrated lime should be prepared separately. Alternatively a copper preparation containing copper oxychloride, carbonate or oxide may be used so long as the copper content is the same as that in the Bordeaux mixture.

Phenyl mercury chloride. Phenyl mercury chloride preparations have been used as satisfactory substitutes for the control of scab on sulphur and copper sensitive varieties. They can only be applied at grower's risk as the physiological behaviour of trees to mercury compounds is incompletely understood.

Neither of the above two materials controls Red Spider (*O. ulmi*) when applied post blossom, so *Derris* or *Lonchocarpus* ground root should be added to the wash to give a rotenone content of 0.004 per cent. The wetters quoted for lime sulphur are suitable. The makers of the mercury preparations should be consulted to ensure that a compatible wetter is used.

Specification of Materials.

Tar Oil Wash. The tar oil wash should contain not less than 3 per cent Grade A or 4 per cent of Grade B high-boiling tar oil.

Petroleum Oil Emulsion. High-boiling petroleum oils conforming to the specification of Grade E are recommended at a concentration of not less than 5 per cent to ensure a satisfactory control of capsid bugs.

Dinitro-ortho-cresol Emulsion. A combined wash of dinitro-ortho-cresol and petroleum oil (Grade E). The wash should be applied at a concentration of 0.1 per cent DNOC and 5 per cent oil for capsid and 3 per cent for spider control. These concentrations are equivalent to $7\frac{1}{2}$ and $4\frac{1}{2}$ per cent respectively of many proprietary washes.

DDT Emulsion. A benzol-DDT-sulphite lye emulsion applied at a concentration of 0.05 per cent DDT, 0.2 per cent benzol. The emulsion is added to water containing a compatible water-soluble wetter, e.g. "Shellestol" 3 pints to 100 gals. wash (0.375 per cent).

Modification for Variation in Pest and Disease Intensity.

Apple Blossom Weevil. It is undesirable to obtain a complete control of this weevil and it is suggested that DDT applied every 4th year will be sufficient in most orchards to ensure a satisfactory level of control. The DDT may be applied at 0.1 per cent with a late applied tar oil. Alternatively the DDT at 0.05 per cent may be added to the late applied petroleum wash.

The DDT-benzol emulsion used as a substitute for winter washing must be applied before the early green cluster stage for the satisfactory control of weevil.

Winter Moth. In some seasons the early instar larva is blown by prevailing winds from infested trees to winter washed orchards. If this occurs it may be necessary to add lead arsenate to the pre-blossom scab washes. If DDT has been applied at bud burst the inblown larvae are generally controlled.

Red Spider. The control of Red Spider by winter petroleum washes in some seasons is disappointing because of the rapid breeding of the mite. The most effective control is obtained from well-applied washes at petalfall, when it is essential to wet completely the under surfaces of the leaves. If severe infestations are expected the addition of rotenone-containing materials to the 3 week wash (No. 6) is recommended. Much depends on the completeness of the wetting of the under surface of the leaves.

Apple Sawfly. The success of the spraying depends on wetting the stamens of the fruitlets before the calyx lobes are erect. It is essential that the inside of the calyx cup is flooded with the nicotine wash.

Codling Moth. If the orchard is subject to severe infestations, a high control can be obtained from an application of DDT wash (0.1%) at the end of the third week in June. This is accompanied by the considerable risk of encouraging a severe infestation of Red Spider, so a compatible rotenone preparation should be included in the DDT wash. This programme is only recommended for one season. In some seasons this wash may coincide with

that given three weeks after petalfall. In this event the DDT may be added to the lime sulphur provided it is compounded in a compatible form.

Scab. The incidence of scab is highest in wet seasons when every effort must be made to apply lime sulphur every ten days. In dry seasons one application may suffice before petalfall and an application at full pink to flowering is the more important. As the flowering stage is approached the concentration of the lime sulphur should be reduced to 1 per cent.

Modification of Programme for Varieties.

Copper sensitive varieties, such as Cox's Orange Pippin, James Grieve, Worcester Pearmain, should not be sprayed with copper fungicides after the green cluster stage.

Stirling Castle is subject to severe sulphur damage at any stage. Lane's Prince Albert is very sensitive after blossoming. The following varieties vary according to district and season in their post blossom sensitivity to sulphur washes: Beauty of Bath, Belle de Boskoop, Lord Grosvenor, Rev. Wilks, St. Cecilia, Blenheim Orange, Charles Ross and Newton Wonder.

Local experience is necessary to determine if they can be sprayed safely and even then there is a risk of severe damage.

Leaf-Eating Weevils (Phyllobius sp.). Occasional severe local infestations are experienced causing damage to the blossom and grafts. A soaking application of a 0.1 per cent DDT wash on the tree and around the base should be given as soon as the infestation is noticed.

II—PEAR.

Stage	Approx. date of spraying	Materials per 100 gal. wash	Pests and Diseases Controlled
1 Bud burst	end March	DDT ($\frac{1}{2}$ lb.) emulsion 2 gal. lime sulphur	Aphis Winter Moth Scab
2 Green cluster	mid April	2 gal. lime sulphur	Scab
3 White bud	end April	1 gal. lime sulphur	Scab
4 Petalfall	mid May	1 gal. lime sulphur	Scab
5 3 weeks after petalfall	end May	1 gal. lime sulphur	Scab

Specification of Materials.

See apple programme.

Alternative Spray Materials.

Copper fungicides or phenyl mercury chloride may be used instead of lime sulphur. (see apple).

Other Control Measures.

Aphis and Winter Moth. If desired a tar oil wash or DNOC petroleum wash (5% oil) winter wash may be used. Wind-borne larvae of the Winter Moth may be controlled by the addition of DDT ($\frac{1}{2}$ lb.) at the white bud stage.

Capsid Bugs. The capsid bugs (*P. rugicollis* and *L. pabulinus*) are both responsible for corky markings on pears. They may be controlled by the

addition of 8 oz. nicotine (95%) and a wetter as recommended for apple at the petalfall stage.

Pear Midge. A heavy application just before bud burst of a tar oil wash at 6 per cent concentration to the area of ground covered by the tree in summer will provide a high control. If the trees are grown in grass it must be kept short and the volume of wash applied sufficient to saturate the turf.

Codling Moth. (See apple.)

Remarks.

The number of applications of fungicidal pre- and post-blossom washes is determined by the intensity of the scab attack; in general, scab of pears is more difficult to keep in check than that of apples. In a dry season application 2 might be omitted and in a wet season it may be necessary to repeat application 5 three weeks later.

The variety Doyenne du Comice is subject to both copper and sulphur injury when sprayed after petalfall. Dr. Jules Guyot is subject to sulphur injury after petalfall.

III—PLUM.

Stage	Approx. date of Spraying	Materials per 100 gal. wash	Pests and Diseases Controlled
1 Dormant	December to mid Feb.	5 gal. DNOC-petroleum emulsion	Aphides Winter Moth Red Spider (some control)
2	May 10-20	Derris (see under) 1 gal. oil emulsion	Plum Sawfly Winter Moth Red Spider
or 2a	end May	1 gal. lime sulphur wetter	Red Spider

Specification of Materials. (See apple.)

Refined oil emulsion. A white oil emulsion for summer use should be prepared with an oil conforming to specification G and complying with the approval scheme of the Ministry of Agriculture.

Derris. *Derris* or *Lonchocarpus* or other rotenone-containing material may be used provided the rotenone content in the wash is not less than 0.004 per cent and in a form that is compatible with the emulsion. A suitable water-soluble wetter may be used instead of the oil but with less satisfactory results. A DDT emulsion may be applied instead of the derris (see apple) either in conjunction with an oil emulsion or with a water-soluble wetter.

Copper Sprays. (See apple.)

Modification for Pests and Diseases.

Red Spider. The stage for effective Plum Sawfly control is sometimes early for satisfactory Red Spider control and it is wise to apply a special spider wash later on varieties very subject to attack. If it is necessary to spray twice it is wise to follow the oil emulsion at stage 2 by derris and wetter in stage 2a, i.e. to avoid the use of oil emulsion and lime sulphur. It should be noted that Early Rivers is sulphur sensitive.

Bacterial Canker. Varieties subject to this disease may be sprayed just after leaf fall with a copper fungicide (0.25 per cent copper) and again just before the opening of the blossom at a lower concentration (0.125 per cent copper).

Silver Leaf. Pruning should be avoided during autumn since the likelihood of infection by silver leaf and bacterial canker is then at its greatest.

Wood Borers. The Scolytid Beetles are not primary pests of the tree, but their presence indicates that the tree is in a low state of vigour due to unsatisfactory soil or manurial conditions or as a result of overcropping or following severe loss of foliage by winter moth or aphid attacks.

IV—CHERRY.

	Stage	Approx. date of spraying	Materials per 100 gal. wash	Pests and Diseases Controlled
1 or 2	Dormant	Dec.—Jan. Dec.—mid Feb.	4-7½ gal. tar oil emulsion 5 gal. DNOC-petroleum emulsion	Aphides Aphides Winter Moth
3	White bud	early April	Copper fungicide (0.125 per cent copper)	Blossom Wilt Bacterial Canker
4	Preceding leaf fall	late October	Copper fungicide (0.25 per cent copper)	Bacterial Canker

Specification of Materials. (See apple).

Modification for Pest and Disease.

Winter Moth. If an infestation develops it may be controlled by the addition of DDT (0.05 per cent) emulsion or dispersible powder to the copper fungicide at the white bud stage.

Cherry Sawfly. The first brood of Cherry Sawfly occurs in early to mid June. It is readily controlled by a derris wash (0.004 per cent rotenone): occasionally a second brood occurs in August.

V—BLACK CURRANT.

	Stage	Approx. date of spraying	Materials per 100 gal. wash	Pests and Diseases Controlled
1 or 1a	Dormant	Dec.—mid-end Feb.	10-12 gal. tar oil petroleum emulsion 7½ gal. DNOC-petroleum emulsion	Aphides, Capsids Winter Moth as above
2	Grape stage	early April	2 gal. lime sulphur ⁽¹⁾	Big Bud Mite
3	Immediately after picking	mid-end July	Copper fungicide (0.2 per cent copper)	Leaf Spot

(¹) 1 gal. for Davidson's Eight.

Specification of Materials. (See apple).

Modification for Pests and Diseases.

Big Bud Mite. Only rooted cuttings entirely free from Big Bud Mite and Reversion should be planted. The plants should be dipped in a winter wash to control capsid immediately before planting. During the first three seasons after planting out they should be sprayed with 5 per cent lime sulphur and DDT (0.1 per cent for the control of Midge) at the grape stage. Subsequently lime sulphur may be applied every third year provided the bushes are grown in an area relatively free from infested crops. This procedure reduces the risk of loss of crop from the combined shock of frost and sulphur.

Leaf-Curling Currant Midge. Vigorous steps to control this pest are essential as soon as it is detected. The winter wash should be applied just before bud burst most liberally to the soil which should be left from the autumn undisturbed by cultivation. DDT (0.1%) should be applied at the grape stage and if necessary a drenching application should be made at a time before the emergence of a fresh brood of the actual midges.

Clay-Coloured Weevil. DDT (0.1 per cent) wash applied as soon as an attack is noticed provides a control. The base of the bush should be liberally sprayed. DDT added to the lime sulphur at the grape stage usually provides a satisfactory control.

Currant Eelworm. There is no satisfactory control and infested bushes should be burned and the land kept free of bushes for some years.

VI—RED & WHITE CURRANTS.

A programme similar to that for black currants is recommended. The concentration of lime sulphur should not exceed 2 per cent.

VII—GOOSEBERRY.

Stage	Approx. date of spraying	Materials per 100 gal. wash	Pests and Diseases Controlled
1 Dormant	Dec.—Feb.	10–12 gal. tar oil-petroleum emulsion	Aphides Capsids Winter Moth
or 1a Dormant	Dec.—Feb.	7½ gal. DNOC-petroleum emulsion	Gooseberry Red Spider
2 Fruit Swelling	End May	Derris-wetter ¾ gal. lime sulphur	Gooseberry Sawfly American Mildew Gooseberry Red Spider

Specification of Materials. (See apple and plum).

Modification for Pests and Diseases.

American Mildew. If commonly prevalent in the plantation it is desirable to apply ¾ gal. lime sulphur per 100 gallons also at the fruit setting stage and 3 weeks later. Many dessert varieties, e.g. Yellow Rough, Early Golden Drop and Leveller are severely defoliated by sulphur preparations: there is no satisfactory method of control on these varieties.

Gooseberry Sawfly. If the spraying at the fruit swelling stage is carried out before the larvae have gone to earth to pupate it should not be necessary to spray again. Derris applied at the end of June or early July will control the second brood.

VIII—RASPBERRY.

Stage	Approx. date of spraying	Materials per 100 gal. wash	Pests and Diseases Controlled
1 Dormant	Dec.—up to late Feb.	4–7½ gal. tar oil emulsion	Aphides Raspberry Moth*
2 Bud burst	mid March	Copper fungicide (0.25 per cent copper)	Cane Spot
3 Pre-flowering	mid May	Copper fungicide (0.125 per cent copper)	Cane Spot
4 10–20 days after full flowering	mid-end June	Derris wash or DDT wash	Raspberry Beetle

* If present, apply tar oil emulsion liberally in late February to soil around the crowns before the soil is disturbed by cultivation.

Specification of Materials. (See apple and plum).

Modification for Pests and Diseases.

Aphis. Frequently winter washing can be omitted.

Raspberry Beetle. Occasionally the beetle may attack the flower buds and cause appreciable damage : in this event it is advisable to apply a derris (0.2 per cent rotenone) or DDT dust (5 per cent).

Cane Spot. May be absent from the beds but at the first sign of infection control measures should be carried out.

Raspberry Cane Midge. If present the base of the canes should be sprayed with benzene hexachloride wash at the beginning and third week in May.

IX—LOGANBERRY.

A programme similar to that for raspberry is recommended. Cane Spot is almost invariably present and routine spraying with copper fungicides should be carried out.

X—BLACKBERRY.

A similar programme to that for raspberry is recommended. Blackberries are subject to severe aphid infestation and therefore they should be tar oil sprayed each season. If capsid is present a 7½ per cent DNOC petroleum wash should be applied before bud burst. Two applications of derris or DDT wash are necessary to control the Raspberry Beetle, the first application ten days after the petalfall stage of the first large batch of flowers and the second two weeks later.

XI—STRAWBERRY.

Stage & Approx. date of spraying	Materials per 100 gal. wash	Pests and Diseases Controlled
1 Obvious crown development in early April	6 oz. nicotine (95%) wetter 1 gal. lime sulphur	Aphides Greenhouse Red Spider Mildew
2 Before flowering in mid May	do.	do.
3 After picking or de-blossoming	do.	do.

It is highly desirable to plant only runners from specially grown parents free from Virus and Tarsonemid Mite.

Whenever it is possible spraying should be replaced by nicotine fumigation. The plants must be subjected to a saturated atmosphere of nicotine at 65°F. for at least one minute for a satisfactory control of aphids.

HETP or TEPP may be used in place of nicotine for spraying provided it is used with every precaution in view of the hazard to the sprayers.

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THE AUTOMATIC SPRAYING OF TOP FRUIT WITH A SPRAY "MAST."

By H. G. H. KEARNS and N. G. MORGAN.

INTRODUCTION.

The high cost of labour, expenditure of physical toil, slowness and lack of uniformity of results associated with the application of fruit tree washes by means of manually operated lances have centred attention on fully mechanised methods of spraying.

The problems involved in spraying are described elsewhere (1). Since then systematic studies on so-called "automatic" spraying have been carried out at Long Ashton. Automatic spraying includes the application of any plant protection material in liquid form, either as small volumes of small droplets of high concentration or of large volumes of large droplets of low concentration, applied until the excess of fluid runs off the target surface.

The application of small volumes of a number of materials to fruit crops presents many technical difficulties which cannot be readily or economically overcome. The eventual development of systemic toxicants safe to plants, consumer and sprayman may overcome some of the difficulties.

It is evident, pending considerably more research, that the interests of fruit growers are best served by recommending that large volumes of "run off" types of washes continue to be used. It is emphasized that a spectacular performance of a spraying machine does not by any means ensure that the wash is applied in a manner that satisfies biological requirements. It cannot be too fully emphasized that the ultimate assessment of a spraying technique must be a biological one (1) and (4). The economics of a spraying technique must be carefully considered and the best compromise must be reached between costs of application and degree of control secured. In some instances it is more profitable to accept a 90 per cent control of a pest or disease than a complete one.

The fruit grower, in contrast to the producer of many other crops, aims at high yields of high grade fruit free from insect and fungus blemishes. Imperfect fruit picked from the bush becomes a major cause of subsequent financial loss.

The control of most insects with large volume ("run off") spraying requires that the target areas of bark, leaf and shoot surfaces, flower buds, flowers, fruitlets, fruit and insects are completely wetted. The application of fungicides is less critical in that complete coverage of the target is not necessary, nevertheless the distribution over the surface of the host plant to be protected must be uniform.

These requirements are extremely exacting and become increasingly difficult to satisfy as a fruit tree increases in height, width and density of wood and foliage. Wind of even 5-7 m.p.h. adds to the difficulties of achieving complete coverage; these difficulties can only be met by increasing the volume of fluid applied per acre.

A skilled sprayman using a "broom" (a multi-nozzle lance) will adjust the rate and method of application to suit the particular make-up of the tree and wind conditions. However, after some hours of spraying, fatigue, especially of the eyes, reduces the thoroughness of the application. The results obtained from another sprayman using the same equipment and volume of wash but

with less skill will provide a markedly lower control of pest and disease. These personal variations can be reduced by selecting the correct spray nozzle performance for a particular type of tree ; nevertheless the variation in results is the most disconcerting problem of lance spraying.

Automatic spraying ensures uniformity in application, whether it be good or bad, as the factors causing variations per given unit area of spray pattern can be controlled. However, automatic spraying takes no heed of individual tree variations. It is therefore necessary to adjust the performance of the machine to give the optimum "coverage" of the most awkward shaped bush or tree.

The up-to-date recommendations for planting orchards insist on wide distances between bushes and rows. This lay-out is ill-suited to efficient automatic spraying. The combination of tall, wide and dense bushes and wide interspaces results in the maximum wastage of spray fluid. The standard tree presents an even more difficult target and it is more economic to use long range spray lances. The difficulties of securing adequate coverage increase rapidly when the tree height exceeds 15 ft., in particular the complete wetting of the under side of leaves becomes almost impossible. The minimum wastage of fluid, highest efficiency of application and simplification of spray machinery is obtained by growing bushes in continuous rows of shallow depth and reasonable height. Since efficient spraying is essential for successful fruit growing and is also the most expensive operation, it would seem that every effort should be made to grow the crop to suit mechanisation.

Large volume automatic fruit spraying machines.

Two methods of applying large volumes of spray fluids have been developed with varying degrees of success, the efficiency depending on the specification of the equipment. The first method depends on breaking up the liquid through nozzles by means of hydraulic flow pressure of 250-500 lbs. per sq. in. which is sufficiently high to give the droplets enough energy to reach the target. The second method, largely developed in the U.S.A., depends on using a large volume of air at very low pressure to carry droplets produced by nozzles operating at about 100 lbs. per sq. in. In either method the best compromise of droplet size is about 1000-3000 microns diameter. The droplets within this size range require considerable energy to propel them to the top of a 15 ft. high tree and through the interspaces between leaves, etc., especially when competing with wind. In most designs of automatic high pressure sprayers the nozzle assembly is arranged behind and not much higher than the spray tank, so that the droplets must be propelled some 10-12ft. to reach the top of 15 ft. high bush trees. Not only must they reach the top of the tree but they must still have sufficient energy to force their way through foliage, etc. It is therefore necessary to adjust the nozzle for long carry and this in turn produces a narrow spray pattern of very large droplets. This results in an unsatisfactory coverage at the top of the tree where it is most desirable to wet everything completely. With this arrangement of nozzles adequate coverage can only be achieved with a considerable and uneconomic wastage of spray fluid.

On trees approximately 15 ft. high the "air flow" automatic machine in which the spray droplets are carried to the target in an air stream provides a considerably better performance, using the same volume of liquid per acre. This is largely due to the fact that droplets of smaller size can be used with this equipment. In addition the rustling effect of the air on the foliage assists in exposing portions of the target to the droplets.

EXPERIMENTAL.

In view of the need for information on the relative efficiencies of spraying by means of spray brooms and high pressure automatic spraying, experimental work was carried out on apples and pears for two seasons. At the time of the trials an "air flow" type of machine was not available.

Various types of nozzle assemblies arranged behind the tank, so greatly favoured by manufacturers of spray machinery, were first examined and none provided an adequate coverage.

Preliminary experiments on the influence of the assemblage of nozzles, spray patterns and volume applied in relation to tree shape were carried out. It was shown that the successful use of high hydraulic pressure on top fruit was largely dependent on arranging the nozzles to discharge droplets of a size range of 1000-3000 micron diameter approximately horizontally into the target. The best assembly consisted of a vertical spray "mast" of nearly the height of the tree: on this nozzles producing suitable spray patterns were fitted. This arrangement produced a coverage of reasonable uniformity, provided 50 per cent of the volume in the form of droplets was able to travel for not less than 75 per cent of the width of the tree when sprayed from both sides on a still day.

Description of experimental machines.

Two tractor trailer spray machines were designed, similar in chassis design, tank capacity, pump drive, haulage and tank filling, but with the following modifications:

(1) *Spray machine with "brooms" (lances)*

High pressure pump	18 gals. per min. delivery.
Number of operators	3 (one tractor driver and two lance operators.)
$\frac{3}{4}$ " B.S.P. nozzles* (with 6/64th in. disc aperture and short range swirl plates)	6 (3 arranged 9" apart on each of two broom heads)
Output per broom	7-8 gals. per min. at 325 lb. per sq. in. flow pressure through 60 ft. of $\frac{1}{2}$ " bore hose.

(2) *Automatic spray machine with "mast"*

High pressure pump	35 gals. per min. delivery.
Number of operators	1 (in tractor cab)
$\frac{3}{4}$ " B.S.P. nozzles* (with 6/64th in. disc aperture and short range swirl plates)	16 (see diagram)
Output per half mast	17 gals. per min. delivery at 250 lb. per sq. in. flow pressure.

The spray mast on the "automatic" machine (see particulars, Figure 1) comprised two $\frac{3}{4}$ " galvanised steel tubes arranged vertically at the rear of the spray machine so that they were adequately supported to withstand both vibration and impact with small branches. The mast was adjustable for height and could be stowed flat on the top of the tank when not in use. The height of the mast for the experiment was arranged so that the top nozzle was 12 ft. from the ground. Sixteen nozzles were fitted, eight on each tube (half mast):

* See refs. (1) and (2).

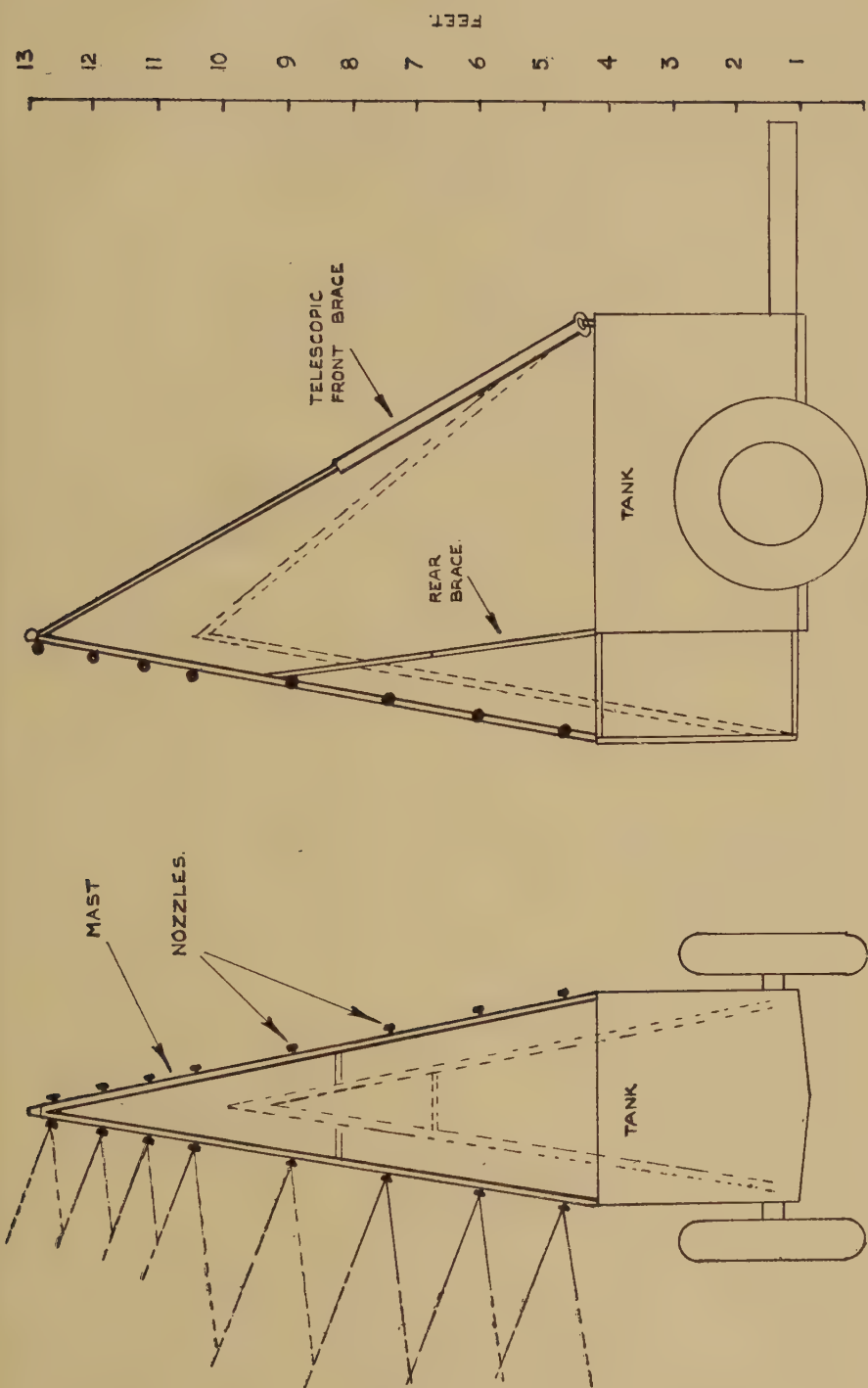


Fig. 1. Automatic "Mast" Fruit Sprayer.
 Diagram showing the assembly of the "mast" on the machine.
 The dotted outline of the mast shows its lowest working position.

the bottom four nozzles were arranged at 18 inches and the top four at 9 inches apart.

The closer spacing of the nozzles at the top of the mast was necessary to compete with wind blowing over and between the thinner vegetation at tree top height. Each tube of the mast had an independent control valve so that one or both sides could be used. The spray pattern was complete along the height of the mast and projected at right angles to the axis of the machine.

Operation.

Apples—(Table I) It is extremely difficult to obtain uniformity of working conditions for field spraying experiments. It was therefore arranged that an 8.6 acre orchard of bush apples of replicated rows of varieties was divided into two approximately equal plots. One plot was sprayed by brooms (lances) and the other by automatic "mast." Each plot was sprayed four times in a year and the trial repeated for two years.

The orchard comprised 15-year old bush trees planted 20 ft. $\times$ 20 ft. The trees were 12–15 ft. high and each row formed a nearly complete avenue or tall "hedge" about 10–12 ft. deep.

Each plot of the orchard was sprayed at the same time and each machine had the same distance to travel within the orchard and for fetching its own spray fluid. The spraying was carried out on days with a wind velocity not exceeding 5 m.p.h.

The automatic "mast" was operated so that two half rows were sprayed at a time with a pump delivery of 35 g.p.m. at 250 lb. per sq. in. flow pressure. A greater flow pressure could not be used as sufficient horse power was not available from the tractor. The speed of the tractor was maintained as far as practicable at $2\frac{1}{2}$ m.p.h.

The performance of the two machines is shown in Table I.

Biological data were recorded.

A summary of the conclusions is as follows :

(1) Approximately the same volume of wash was applied by the two methods of application.

(2) The automatic "mast" applied the spray fluid in half the time at approximately one-fifth the expenditure of labour and at half the cost of tractor fuel.

(3) The use of the automatic machine saved considerable physical toil.

(4) The control of aphids obtained by both machines by the application of D.D.T. benzol emulsion at bud burst stage was nearly complete.

(5) There was no significant difference in the control of apple scab.

(6) Hand spraying with brooms (lances) provided a better control of Red Spider than the automatic machine due to the former providing a better coverage of the under surface of the leaves.

Pears (Table II). A trial was carried out on approximately 4 acres of bush pears planted 20 ft. $\times$ 15 ft. and 9–12 ft. high. The bushes were more compact than the apples and provided an easy target for the automatic "mast." It was not possible to arrange that "broom" (lance) and automatic spraying was carried out in the same season. However, for all practical purposes the conditions of operation were similar in the two consecutive years used for the trial. The plot is exposed to a frequent prevailing wind and the opportunity was taken to determine if spraying into the wind from one tube of the mast (8 nozzles) would spray adequately one row of bushes at a time. This method was satisfactory and provided an appreciable saving in wash used per acre

when compared with brooms (lances). In 1948 an exceptionally severe infestation of aphid was controlled by a D.D.T. benzol emulsion. The control of scab was very high.

TABLE II.

COMPARISON OF "SPRAY BROOM" AND AUTOMATIC "MAST" SPRAYING. 3.8 AC. BUSH PEAR TREES 12 FT. HIGH. SPACING 15 FT. $\times$ 12 FT. (242 BUSHES PER AC).

Spray stage	1947				1948			
	Spray Broom (1)				Mast (2)			
	Total gals.	Gals. per ac.	Operating time; hours & minutes	Total man hours	Total gals.	Gals. per ac.	Operating time; hours & minutes	Total man hours
Dormant	1770	465	8-7	16-14	—	—	—	—
Green bud	2205	580	7-37	22-51	1150	302	2-47	2-47
White bud	1880	490	7-10	21-30	1125	290	2-57	2-57
Petal-fall	2060	542	6-14	18-42	1000	263	2-24	2-24
3 weeks	2032	534	—	—	900	236	2-15	2-15

Total hours include all operations in the orchard.

Speed of automatic machine—2-2½ m.p.h.

Distance travelled by automatic machine per acre—968 yds. approx.

Notes: (1) 2 3-nozzle brooms.

(2) 8-nozzle "mast."

SUMMARY

(1) Automatic spraying of top fruit by means of high hydraulic pressure is satisfactory provided the nozzle assembly, delivery of the pump and "make-up" of the tree are suitable.

(2) A spray mast of a height equal, or nearly equal, to the height of the tree is essential: such an assembly is not suited to many existing orchards.

(3) For application of spray material from one side only of the machine, to bush trees of not more than about 15 ft. high, the delivery of the pump must not be less than 20 gallons per minute at a flow pressure of at least 250 lb. per sq. in.

ACKNOWLEDGMENT.

Thanks are due to Miss C. E. C. Clarke who assisted in the assembly of the machines, ensured their efficient operation in the orchard and recorded their performance.

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A LIGHTWEIGHT SPRAY NOZZLE ($\frac{1}{4}$ " B.S.P.) FOR "SMALL" AND "LARGE" VOLUME SPRAYING.

By H. G. H. KEARNS and N. G. MORGAN.

The development during the past few years of hormone weed killers and systemic insecticides has centred attention on methods of application. Small volumes of high concentration may be used instead of large volumes of low concentration. The absorption and translocation within the plant of these materials is particularly favourable for the successful use of small volumes, as incomplete coverage with spray droplets is often sufficient. Some of the new organic insecticides of very high toxicity and some fungicides of relatively low phytotoxicity are also suitable for small volume spraying on some targets.

The application of small volumes requires nozzles or other equipment capable of forming readily reproducible spray patterns of small droplets. The most satisfactory method of "atomising" a liquid into droplets is by forcing it through a nozzle by hydraulic pressure.

The delivery of a nozzle varies both in volume and size of droplet; it may be a few fluid ounces to gallons per minute, with droplet size ranging from a few to several thousand microns in diameter. The design and operating conditions of the nozzle determine the performance.

In view of the importance of obtaining an economic and satisfactory use of expensive spray fluids it is not surprising that there are numerous designs of nozzles. Most of these appear to have been made without any understanding of the factors (1) governing their performance, and therefore the significance of small deviations and errors in manufacture have not been appreciated. Lack of uniformity in performance is a common feature. The most successful design of nozzle, for a number of practical reasons, is the swirl type, in which the fluid under pressure enters tangentially into a chamber and is expelled under centrifugal force through a round aperture.

On the majority of the more simple spraying equipment such as knapsacks, manually operated pumps, etc., the swirl nozzle is made in its simplest form. Often it consists of a brass cap screwed on to a swirl chamber with a tangentially drilled hole in the wall. The workmanship and performance is generally poor. The most satisfactory design consists of a nozzle body and cap in which swirl plate and disc can be changed to provide a wide range of performances. This design has been developed with various modifications (2) for the application of large volumes and is of relatively large size, well suited for many field uses. The nozzle can be modified to apply "small" volumes but for this purpose it is unnecessarily large, particularly for use on light lances.

The greatest progress in small ("low") volume spraying with weed killing hormones has been made in U.S.A. Two principal types of nozzle have been developed; one is an expensive precision-made swirl type and a second a more simple nozzle producing a batwing shaped spray pattern (so-called flat spray). The latter type requires a high standard of precision manufacture and some designs are subject to very rapid abrasion if particulate materials are sprayed. Ceramic flat spray nozzles are also available (3). A "flat spray" pattern has no advantage over the round type produced by a swirl nozzle.

A simple lightweight small volume nozzle has been designed to facilitate cheap manufacture and to ensure a performance sufficiently uniform for

practical purposes. The nozzle may also be used for large volume ("run off") spraying by a simple interchange of cheaply made parts.

The Design.

Details of the design are shown in the drawing (Fig. 1). The nozzle comprises a body, nozzle cap and gasket, filter, swirl chamber plate and disc. The body comprises a hexagonal nipple with a parallel $1/4"$ B.S.P. free fit thread of equal length on either side of a $5/16"$ W. hexagonal (A.F. $19/32"$). The two ends of the body are machined flat and at right angles to its axis. The body is bored centrally with $11/32"$ hole; each end is accurately bored $3/8"$ wide and $5/32"$ deep to form clean cut ledges at right angles to the bore. The bored recess at one end accommodates a filter and that at the other end a swirl chamber plate and disc assembly.

The filter comprises a round disc of phosphor bronze gauze with the apertures smaller than that of the spray disc. The disc is held in position against the ledge by a brass retainer ring $3/8" \times 1/16"$ made of 18 G. tube and it may be split longitudinally and slightly opened to form a spring fit, or alternatively a complete ring may be used. In the latter case the gauze is soldered to the ring or the ring inserted as a tight fit to wedge against the gauze disc.

The cap is a $5/16"$ W. hexagonal (A.F. $19/32"$) $5/16"$ long and tapped $1/4"$ B.S.P.; the closed end is bored centrally with a $11/32"$ round hole; the ledge formed by the hole must not exceed $1/16"$ thickness. The inside surface of the ledge must be machined flat and at right angles to the thread to accommodate a nozzle gasket of lead or a moderately hard oil resistant synthetic rubber, $15/32" \times 11/32" \times 1/32"$.

The swirl chamber assembly is in two forms—one type for small ("low") volume spraying of concentrates, a second for the application of "run off" type of spray fluids.

The assembly for "small" volume spraying comprises a swirl chamber plate and disc. The swirl chamber plate is a solid brass plate $3/8"$ wide $\times 1/8"$ thick; the $3/8"$ is made a sliding fit by removal of a few thousandths of an inch from the diameter and the plate is bored to form a cylindrical swirl chamber $1/4"$ diameter $\times 1/32"$ deep. A horizontal slot $1/64"$ wide and $1/32"$ deep is cut from the circumference of the plate tangentially to the edge of the chamber; the slot from the edge of the swirl plate is extended through the depth for $1/16"$; care must be taken to avoid cutting into the floor of the chamber. The vertical slot must clear the ledge on which the swirl plate rests to provide a portway of larger surface area than the aperture of the disc.

The disc is a circular plate $3/8"$ diameter $\times 1/32"$ thick and accurately drilled in the centre with a $2/64"$ hole. The disc is made a sliding fit by removal of a few thousandths of an inch from the diameter. The hole must be at right angles to the width of the plate, with the wall of the hole clean and edges free from all burrs. The disc is fitted on top of the swirl chamber plate and the whole assembly held together to make a watertight joint by screwing down the nozzle cap on the lead or rubber gasket.

The assembly for large volume ("run off") spraying comprises a swirl plate $3/8"$ (sliding fit) $\times 1/16"$ with two opposite side slots $1/16"$ wide and $3/32"$ deep cut at an angle of 45° . A swirl chamber is formed by inserting a swirl chamber ring $3/8"$ wide $\times 1/16"$ deep made out of 18 G. brass tube.

The nozzle is screwed into a lance adapter tapped $1/4"$ B.S.P., or into saddles tapped $1/4"$ on spray booms and brooms. A watertight seal is made

between the nozzle body and adapter or saddle by means of oil-impregnated fibre, lead or leather washer $11/16'' \times 1/2'' \times 1/16''$.

Performance.

The nozzle in both "small" and "large" volume assemblies is designed to operate at 40–75 lbs. per sq. in. pressures. Experience has demonstrated that for many purposes a pressure of 75 lbs. per sq. in. avoids many difficulties met under field conditions.

The nozzle may be used singly on a lance, in numbers on a straight boom, or arranged on rings for use on machines using a flow of air as a means of conveying the droplets to a target.

The small volume nozzle assembly was designed to produce a reasonably high percentage of droplets of a size range from 150–250 microns diameter, a size that is a compromise for use under a wide range of field conditions. The output with a $2/64$ th" disc aperture at 75 lbs. per sq. in. pressure is 8 fl. oz. per minute approximately and the best spacing of nozzles on booms is nine inches apart.

The filter on the nozzle is only fitted as a final precaution against blockage of the disc aperture.

The "large volume" nozzle assembly may be used with 3-, 4- or $5/64$ th in. disc apertures. With these apertures the approximate delivery at 75 lbs. per sq. in. pressure is 35, 80 and 90 fl. oz. per minute respectively. The swirl plate described is a compromise in design to give a satisfactory performance with the three apertures. The best spacing between nozzles on booms is $13\frac{1}{2}$ inches. The nozzle filter may be omitted with apertures above $2/64$ th in.

The nozzle may be made entirely of brass of a total weight of $\frac{3}{4}$ oz. approx. An abrasion and corrosion resistant steel or bronze disc is preferable to brass. The nozzle body may be made of duralumin but this material is corroded by many copper-containing fluids. A duralumin cap is not satisfactory when screwed to a duralumin body as the threads corrode and seize unless kept lubricated with oil.

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THE RÔLE OF NITROGEN IN FERMENTATION.

III. FURTHER OBSERVATIONS ON THE NITROGEN CHANGES WHICH OCCUR DURING FERMENTATION AND PRELIMINARY OBSERVATIONS ON THE ASSOCIATED CHANGES IN PHOSPHORUS.

By L. F. BURROUGHS and S. W. CHALLINOR.

In a previous paper (1) the fermentation of a Bramley juice was studied with particular reference to the changes in the nitrogen content of the juice and the associated changes in specific gravity which occurred as the fermentation proceeded. The analytical results showing the magnitude of these changes in the nitrogen content of the juice were converted into data representative of the growth and distribution of yeast which occurred during the fermentation. It was shown that utilisation of the "soluble nitrogen" by the yeasts was rapid and that "soluble nitrogen" reached its minimum in only seven to eight days although fermentation continued during forty days. The "soluble nitrogen" which was available to and utilised by the yeasts was equal in amount to approximately 80% of the "soluble nitrogen" originally present in the juice.

In the work described in a subsequent paper (2) the fermentation of the same Bramley juice was checked at different specific gravities by centrifuging and the stability of the resulting ciders was examined. Varying degrees of stability were obtained and an attempt was made to explain the *mechanism* of this method of controlling fermentation, by a study of the changes in the nitrogen content of the fermenting juices which occurred prior to centrifuging and during storage of the ciders.

It was shown that effective control of the fermentation may be expected if the juice is centrifuged *after utilisation of the readily available "soluble nitrogen" is complete* but that some factor other than nitrogen might also be involved. It was suggested that this other factor might be phosphorus.

The present investigation is a continuation of the work described in papers (1) and (2). Two main objects were in view, namely, to obtain a more complete understanding (a) of the various factors which determine the course of fermentation and (b) of the mechanism of the "Fermentation Control of Ciders by the Centrifuge Method" (2).

Apples of the Bramley variety were again chosen and eight casks of the juice were available. The nitrogen content of this juice was much higher than that of the Bramley juice previously used. Fermentation in one of these casks was allowed to proceed without interference, i.e. was not checked in any way, whereas the fermentations in the other seven casks were checked at different specific gravities by centrifuging. An investigation was made during each of these fermentations of the changes which occurred in the fermenting juice with respect to its nitrogen and phosphorus contents and the associated changes in its specific gravity and sugar content.

Experimental work involving centrifuging is still in progress and will be described in a subsequent report. The present paper is concerned entirely with the changes which occurred in the juice in which the fermentation was undisturbed.

EXPERIMENTAL.

Processing and Sampling Procedure.

Approximately three tons of Bramley apples were washed, milled and pressed (13/12/1949), yielding 450 gallons of juice which was bulked in a slate tank. The juice was thoroughly mixed to ensure uniformity and was transferred to a series of eight casks (mainly hogsheads) and also into jars, in such a way that the juice in each cask and jar was representative of the bulked juice. All vessels used had previously been treated with sulphur dioxide and thoroughly cleaned.

The casks and jars were placed in the cider house and fermentation allowed to proceed. As explained in the introduction, in this paper we are concerned only with cask 1 (a hogshead) in which the fermentation was allowed to proceed undisturbed. When the "brown head" had ceased to form, the cask (and its accompanying jars) were cleaned and fitted with a fermentation lock.

Samples of the fermenting juice for laboratory examination were syphoned from the centre of the cask at daily intervals, as far as practicable, during the first month and subsequently at progressively longer intervals.

Frequent "topping-up" of the cask was required as a result of the withdrawal of samples and of the other losses incidental to the use of this type of container. The reserve of fermenting juice (three gallons) in the accompanying jars was not sufficient for all the "topping up" required and after about three months the cider in the cask was covered with a thin layer of liquid paraffin to prevent access of air to the cider and possible acetification. In order that samples of cider could be withdrawn without contamination with liquid paraffin a syphon was fitted to the cask.

Analytical Methods.

A very brief outline of the more important stages in the analytical methods used in this investigation is given below, with the appropriate references.

Specific Gravity. The specific gravities of the juices and ciders were determined by hydrometer (N.P.L. tested) and corrected to a temperature of 15°C. The samples were degassed and, when necessary, filtered before S.G. determination.

Sugars. Samples (25 ml.) of juice or cider were neutralised with excess CaCO_3 (0.5 gm.) and preserved by addition of alcohol (100 ml.) until required for analysis. Suitable aliquots* of the clear supernatant were evaporated to remove alcohol, clarified with 5% $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ solution and 0.3N. Ba(OH)_2 , according to Somogyi's method (3), made up to 100 ml. and filtered. A suitable aliquot* of the filtrate was diluted to 100 ml. and the reducing sugars present in this solution (*before* inversion with invertase) were determined by Somogyi's method (4). Reducing sugars *after* inversion (with invertase at pH 4.6) were determined on another similar aliquot of the filtrate. Reducing sugars (*before* and *after* inversion) are expressed in terms of invert-sugar (g./100 ml.) and sucrose is calculated from the difference between reducing sugars present *before* and *after* inversion.

There is a possibility that non-reducing sugars other than sucrose, but with the same glucose-fructose linkage as sucrose (e.g. raffinose) may have been present in the juice. If such non-reducing sugars were present

* Different combinations of dilutions were used according to the specific gravity of the juice or cider.

they would have been partly hydrolysed by the invertase used, with liberation of reducing sugars (e.g. fructose and mellibiose from raffinose) and would therefore be estimated as sucrose by the analytical methods employed.

Nitrogen. The method used for the determination of nitrogen differs in two important respects from that described previously (1). In the present method (a) the micro-Kjeldahl digestion was continued for eight hours after clearing, and (b) the whole of the digest solution was transferred to the Markham distillation apparatus. A suitable aliquot (2-5 ml.) of sample which had been acidified with 1/10th of its volume of 11 N. H_2SO_4 was evaporated to a black syrup in the presence of 0.5 ml. H_2O_2 (100 vol.) and a few pieces of 20-mesh carborundum to prevent bumping (5). Sulphuric acid (2-3 ml.) and catalyst (0.5 g.) (6) were then added and heating was continued until the digest was clear and for a further eight hours. The digest solution was finally transferred into the Markham distillation apparatus and the ammonia present was determined as described previously (1). All determinations were made in duplicate.

Phosphorus. The same acidified samples of juice or cider used for nitrogen determination were also used for the determination of phosphorus. A wet-digestion procedure based on that of Alleroft and Green (7) was used; an aliquot (5 or 10 ml.) of the sample was heated in a Pyrex boiling tube with 60% perchloric acid (2 ml.) and conc. nitric acid (1 ml.) (a few pieces of 20 mesh carborundum were added to prevent bumping). The mixture was heated to charring and until white fumes appeared. After cooling slightly, a further portion of conc. HNO_3 (1-2 ml.) was added and the digestion continued until all organic matter was destroyed. The digest solution was then transferred to a graduated flask, neutralised to phenolphthalein with N.NaOH and made up to 100 ml.

The phosphorus content of this solution was determined by Holman's method (8) in which acidified KI is used as the reducing agent and the liberated iodine discharged with Na_2SO_3 solution leaving a clear blue solution. The intensity of the blue colour of this solution was measured on a Spekker Absorptiometer using an orange filter (Ilford 607) and the phosphorus content of the solution was calculated by comparison with a standard curve.

Both "soluble phosphorus" and "soluble nitrogen" were determined on samples of juice or cider which had been freed from suspended material by *filtration*. A small quantity (approx. 1 g./100 ml.) of Kieselguhr was added to the degassed sample which was then filtered under reduced pressure. The Kieselguhr ("Hyflo") used for this filtration had previously been treated to remove any acid-soluble nitrogen and phosphorus.

In the two previous papers (1 and 2) "soluble nitrogen" was determined after centrifuging at 2,500 r.p.m. for 30 minutes. The relative efficacy of centrifuging and filtering are indicated by the following determinations made on the sample of bulked-juice.

				mg. N/100 ml.	mg. P/100 ml.
Untreated juice	..	..	..	27.9	5.75
Centrifuged juice	..	..	..	27.0	5.70
(2,500 r.p.m. for 30 mins.)					
Once filtered juice	..	..	..	26.2	5.55
Twice ,,	..	..	..	26.1	5.55

The once-filtered juice was filtered as described above and the twice-filtered juice received a second filtration using a large excess (10 g./100ml.)

of Kieselguhr. The results show that there was no appreciable adsorption of either nitrogen or phosphorus from solution by the Kieselguhr.

RESULTS.

The composition and properties of the bulked juice and of the juice in cask 1 are summarised below. It will be seen that a high degree of uniformity was achieved since differences between the two samples of 'juice' were only slight and were in fact of the order of the experimental errors involved in the respective determinations.

	<i>Bulked juice</i>	<i>Juice in Cask 1</i>
Specific gravity	1051½	1051
Reducing sugars after inversion (expressed as invert sugar g./100 ml.)	10.80	10.70
Reducing sugars before inversion (expressed as invert sugar g./100 ml.) ..	8.50	8.40
"Sucrose" (g./100 ml.)	2.20	2.20
Titrateable acidity (as malic acid g./100 ml.)	0.96	—
pH	3.16	—
"Tannin" (i.e. permanganate-reducing substances)	0.07	—
"Total nitrogen" mg.N/100 ml. ..	27.9	28.1
"Soluble nitrogen"	26.2	25.8
"Total phosphorus" mg.P/100 ml. ..	5.75	5.75
"Soluble phosphorus"	5.55	5.50
*Rate of Fermentation at 25°C. ..	*10.0	*9.0

As stated in the introduction this work is a continuation of the study of the fermentation of apple juice which was described in the first paper of this series (1). Comparisons are made of the changes in nitrogen content and of the associated changes in specific gravity which occurred during the fermentation in cask 1 and that in pipe F of the previous investigation (1). No determinations were made of the sugar content and the phosphorus content of the juice in pipe F.

The Fermentation.

The juice in cask 1 was allowed to ferment to dryness. The fermentation followed its natural course and no attempt was made to check the fermentation (e.g. by centrifuging) at any intermediate stage. Observations on the changes which occurred as the result of autolysis of yeast were required and the dry cider was therefore allowed to remain on its lees after fermentation had ceased. Samples of the fermenting juice or cider were removed at intervals for laboratory examination and changes in (i) specific gravity and sugar content (ii) "nitrogen" content (iii) "phosphorus" content were recorded during both the fermentation and storage period and are described below.

(1) Changes in Specific Gravity and Sugar Content of the Juice During the Fermentation.

The results showing the decrease in specific gravity and in the sugar content of the fermenting juice are summarised in Table I and are plotted in Fig. 1.

* Determined on laboratory samples at 25°C; the fermentation in Cask 1 took place at cider house temperature.

TABLE I.

CHANGES IN SPECIFIC GRAVITY AND IN SUGAR CONTENT OF THE JUICE IN CASK 1 DURING
FERMENTATION AND STORAGE.

(ALL SUGAR CONTENTS ARE EXPRESSED AS g. SUGAR/100 ml. OF JUICE OR CIDER AND
ARE CORRECTED TO THE NEAREST 0.05 g.)

Duration of Fermentation and Storage. Days	Specific Gravity	Reducing Sugars as invert-sugar (g./100 ml.)		"Sucrose" (g./100 ml.)
		After inversion	Before inversion	
0	1,051	10.70	8.40	2.20
2	1,051 $\frac{1}{2}$	—	—	—
3	1,051	10.50	8.50	1.90
5	1,050 $\frac{1}{2}$	10.25	8.50	1.70
6	1,049	10.05	8.40	1.55
8	1,046	9.40	8.45	0.90
10	1,043	8.75	8.30	0.45
14	1,031	6.25	6.15	0.10
15	1,027 $\frac{1}{2}$	5.25	5.25	0.05
16	1,022	4.30	4.30	0.05
17	1,019 $\frac{1}{2}$	—	—	—
18	1,017 $\frac{1}{2}$	3.30	3.25	0.05
20	1,014	2.50	2.45	0.05
21	1,012	—	—	—
23	1,009	—	—	—
24	1,008	1.20	1.15	0.05
25	1,007	—	—	—
27	1,005 $\frac{1}{2}$	—	—	—
28	1,005	—	—	—
29	1,004	—	—	—
30	1,004	0.40	0.40	0.00
31	1,004	—	—	—
34	1,003	—	—	—
45	1,003	0.20	0.20	0.00
55	1,003	0.15	0.15	0.00
76	1,003	0.15	0.15	0.00
104	1,003	0.15	0.15	0.00
132	1,003	0.15	0.15	0.00

There was an initial lag-period of five days during which only half of a degree (approx.) of specific gravity was lost. After the 5th day the rate of fermentation (i.e. rate of decrease of S.G.) gradually became more rapid and was at its maximum (4.5° per day) between the 14th and 16th days when the S.G. was in the range 1,030–1,025. After the 16th day the rate of fermentation began to decrease although it was still fairly rapid up to the 25th day (S.G. 1,007). Between the 25th and 33rd days fermentation became progressively slower and after the 35th day no decrease in S.G. was recorded even on prolonged storage (approx. three months).

The graph showing amount of reducing sugars in the fermenting juice (after inversion with invertase) is similar in shape to that showing the change in specific gravity. It is not possible here to discuss fully the relation between the changes in specific gravity and the changes in sugar content which occurred during the fermentation. The decrease in gravity of the juice which occurred during fermentation was largely due to the disappearance of sugar but the gravity is also affected by dissolved solids other than sugar and by the alcohol produced during the fermentation.

The observations on the changes in the sugar content of the fermenting juice show several points of interest, namely :

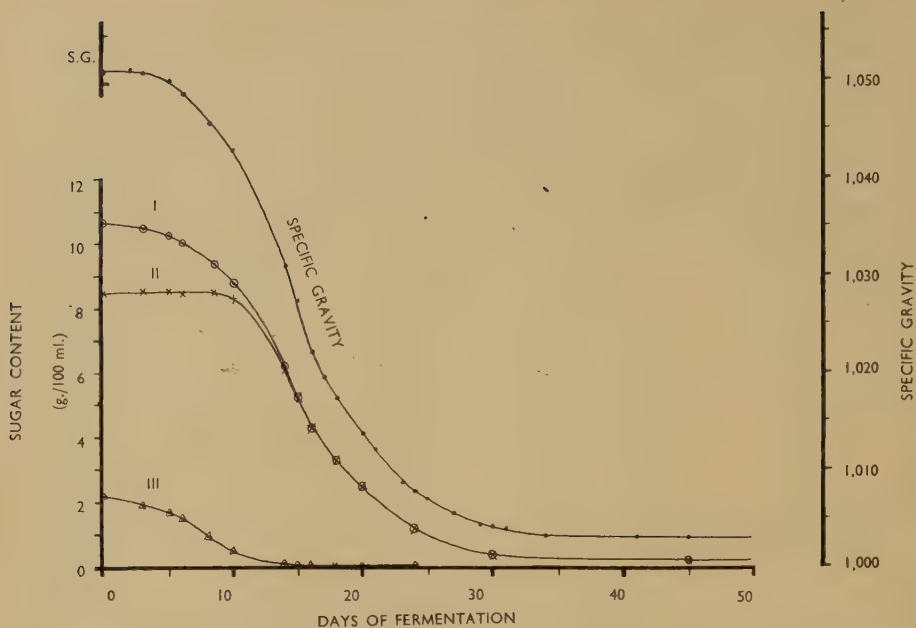


Fig. 1. Changes in Specific Gravity and in Sugar Content of the Juice in Cask I during Fermentation and Storage.

Curve I - Reducing sugars *after* inversion (as invert-sugar).

„ II - Reducing sugars *before* inversion (as invert-sugar).

„ III - "Sucrose."

(a) During the first five days (the lag-period of fermentation) the sugar content decreased by 0.45 g./100 ml. whereas the specific gravity decreased by only half a degree.

(b) The amount of reducing sugars present in the fermenting juice, remained fairly constant during the first ten days. Since, however, an appreciable amount (approximately 2 g./100 ml.) of sugar was fermented, it follows that reducing sugars were being produced, presumably from sucrose, at a rate approximately equal to that at which they were being fermented.

(c) In the fresh juice, the amount of sucrose present (2.20 g./100 ml.) was 20.6% of the total reducing sugars present after inversion with invertase, expressed as invert-sugar (10.70 g./100 ml.). Hydrolysis during fermentation was, however, rapid and by the 15th day sucrose had practically disappeared from the fermenting juice.

(2) Changes in the Nitrogen Content of the Juice During Fermentation.

We are here concerned with nitrogen (a) in the compounds in solution ("soluble nitrogen" i.e. "nitrogen in solution") (b) in the material in suspension ("nitrogen in suspension") and (c) both in solution and in suspension ("total nitrogen.") "*Nitrogen in the compounds in solution*" was determined on the sample of juice or cider after filtration (see Analytical Methods). "*Total Nitrogen*" was determined on the sample of juice or cider directly, after shaking to disperse any material which had settled out, i.e. it includes the "nitrogen" both in solution and in suspension but *not* that in the lees. "*Nitrogen in the material in suspension*" in any particular sample was determined by calculation—it is represented by the difference between the "soluble nitrogen" and the "total nitrogen" of that sample.

As explained in the previous paper (1), the changes in the relative amounts of these different forms of "nitrogen" which occurred during fermentation can be converted into data representative of the growth and distribution of yeast during fermentation. Such interpretation of the nitrogen-data has been made when necessary in this paper—in particular in the section dealing with "nitrogen in suspension."

The results showing the changes in the nitrogen content of the juice during fermentation in cask 1 are summarised in Table II and are graphed in Fig. 2. All results are expressed as mg. N/100 ml. of juice or cider and in the text below are abbreviated to milligrams (mg.).

(a) *Nitrogen in the Compounds in Solution.*

During fermentation the "soluble nitrogen" content of the juice *decreased* from 25.8 to 8.7 mg., i.e. to 33.6% of its initial value during 24 days. This decrease (17.1 mg.) is indicative of yeast growth and it will be seen that there was a heavy growth of yeast during this time.

The time during which this decrease in "soluble nitrogen" occurred could

TABLE II.

CHANGES IN THE NITROGEN CONTENT OF THE JUICE IN CASK 1 DURING FERMENTATION AND STORAGE.

(ALL RESULTS FOR NITROGEN ARE EXPRESSED AS mg. N/100 ml. OF JUICE OR CIDER AND ARE CORRECTED TO THE NEAREST 0.1 mg.)

Duration of Fermentation and Storage. Days	Specific Gravity	Nitrogen in the Fermenting Juice or Cider mg.N/100 ml.)		
		Total Nitrogen	Nitrogen in the Compounds in Solution	Nitrogen in the Material in Suspension
0	1,051	28.1	25.8	2.3
2	1,051½	27.4	25.8	1.6
4	1,051	25.7	25.3	0.4
6	1,049	24.4	24.2	0.2
8	1,046	23.3	21.8	1.5
10	1,043	22.5	18.6	3.9
14	1,031	22.4	13.2	9.2
15	1,027½	22.2	11.8	10.4
16	1,022	21.4	10.7	10.7
17	1,019½	20.2	10.4	9.8
18	1,017½	19.0	9.8	9.2
20	1,014	—	9.6	—
21	1,012	16.4	9.1	7.3
23	1,009	14.2	9.1	5.1
24	1,008	—	8.7	—
25	1,007	—	8.7	—
27	1,005½	11.9	8.7	3.2
28	1,005	—	8.9	—
29	1,004	11.2	8.9	2.3
30	1,004	—	9.2	—
31	1,004	10.5	9.3	1.2
34	1,003	10.2	9.5	0.7
36	1,003	—	9.7	—
38	1,003	—	9.7	—
41	1,003	10.1	10.0	0.1
45	1,003	—	10.1	—
55	1,003	10.6	10.3	0.3
76	1,003	11.0	10.8	0.2
104	1,003	11.6	11.3	0.3
132	1,003	11.7	11.6	0.1

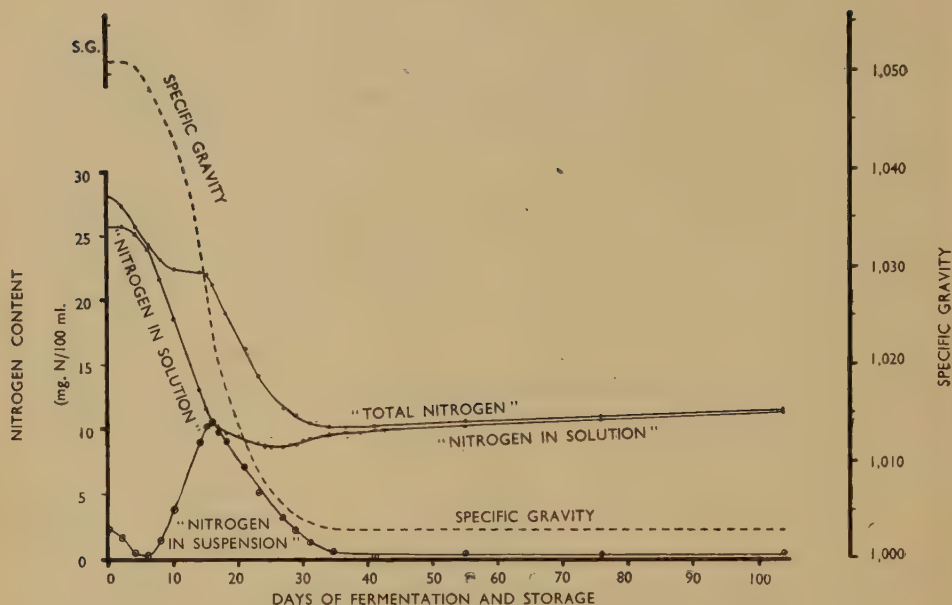


Fig. 2. Changes in the Nitrogen Content of the Juice in Cask 1 during Fermentation and Storage.

be divided into three distinct periods. Thus, there was no appreciable decrease during the first four days (lag-period); from the 6th day there was a rapid decrease until the 16th day (a period of rapid growth of yeast); after the 16th day "soluble nitrogen" decreased progressively more slowly until the 24th day. The *minimum* value for "soluble nitrogen" (8.7 mg.) was reached about the 24th day. At this time the amount of reducing sugars present (both before and after inversion with invertase) was 1.20 g./100 ml. and the specific gravity was 1,008.

After the 27th day "soluble nitrogen" *increased* markedly, due presumably to the autolysis of yeast (see later). Between the 27th and 41st days "soluble nitrogen" increased from 8.7 to 10.0 mg. and the rate of increase was relatively rapid; soon after the 41st day the rate became slower and remained constant over the next three months; the value reached on the 132nd day (24/4/1950) was 11.6 mg. This increase from 8.7 to 11.6, i.e. 2.9 mg., is equivalent to 33.3% of the minimum "soluble nitrogen" recorded (8.7 mg.).

(b) "Total Nitrogen."

During the first four days, "total nitrogen" decreased from 28.1 to 25.7 mg. and, since the "soluble nitrogen" remained constant during this time, it follows that sedimentation of nitrogenous material must have occurred.

The chief point of interest in the graph for "total nitrogen" (Fig. 2) is that there was no appreciable decrease between the 10th and 15th days although during this time the "soluble nitrogen" content of the juice decreased rapidly. It follows therefore that fermentation was vigorous enough to prevent accumulation of yeast in the lees and that the amount of yeast which was maintained in suspension was equivalent to the amount which grew during this time. In this connexion it should be borne in mind that the relative distribution of

yeast is probably continually changing as a result of the interchange of yeast between that in the lees and that in suspension.

After the 15th day "total nitrogen" decreased rapidly but did not reach its minimum (10.1 mg.) until some time between 34th and 41st days when fermentation had practically ceased (S.G. 1,003).

After the 41st day there was an *increase* in "total nitrogen" and this may be explained by the increase which took place in the "soluble nitrogen" content of the cider as the result of autolysis of the yeast.

(c) *Nitrogen in the Material in Suspension.*

As mentioned in the beginning of this section "nitrogen" in the material in suspension is taken as representative of the yeast in suspension.

"Nitrogen in suspension" *decreased* during the first four days coinciding with the lag-period of fermentation during which time "soluble nitrogen" and specific gravity remained approximately constant. The material which sedimented during this time, however, was not entirely yeast since particles of apple tissue present in the fresh juice would also be deposited. No increase in "nitrogen in suspension" occurred until after the 6th day when it started to *increase* rapidly and at a constant rate. Observations with the microscope on the fermenting juice showed that the material in suspension during this period was almost entirely yeast and only traces of other forms of suspended material were observed.

The *peak value* of "nitrogen in suspension" occurred about the 16th day at which time the specific gravity was 1,022. Between the 16th and 27th days "nitrogen in suspension" *decreased* rapidly at a rate almost as great as that at which it had increased (between the 6th and 16th days). From the 27th to the 34th days the rate of decrease was rather slower but was still rapid; on the 34th day the amount of "nitrogen in suspension" was very small (0.7 mg.) and during the next three months it decreased further.

(3) **Changes in the Phosphorus Content of the Juice During Fermentation.**

We are here concerned with phosphorus (a) in the compounds in solution ("soluble phosphorus" i.e. "phosphorus in solution"), (b) in the material in suspension ("phosphorus in suspension") and (c) both in solution and in suspension ("total phosphorus"). These terms correspond with those used in the description of the changes in nitrogen.

"*Phosphorus in the compounds in solution*" was determined on the sample of juice or cider after filtration (see Analytical Methods). "*Total phosphorus*" was determined directly on the juice or cider after shaking the sample to disperse any material which had settled out, i.e. it includes both "soluble phosphorus" and "phosphorus in suspension" but *not* "phosphorus in the lees." The amount of "*phosphorus in the material in suspension*" in any particular sample was obtained by calculation—it is represented by the difference between the "soluble phosphorus" and the "total phosphorus" of that sample.

The analytical results illustrating the changes in the phosphorus content of the juice during fermentation are given in Table III and are graphed in Fig. 3. The changes in these phosphorus contents followed a general trend which was closely similar to that observed in the changes in the corresponding nitrogen contents (Table II and Fig. 2). All results for phosphorus are expressed as mg.P/100 ml. of juice or cider and in the text below are abbreviated to milligrams (mg.).

TABLE III.

CHANGES IN THE PHOSPHORUS CONTENT OF THE JUICE IN CASK I DURING FERMENTATION AND STORAGE.

(ALL RESULTS FOR PHOSPHORUS ARE EXPRESSED AS mg. P/100 ml. OF JUICE OR CIDER AND ARE CORRECTED TO THE NEAREST 0.05 mg.)

Duration of Fermentation and Storage : Days	Specific Gravity	Phosphorus in the Fermenting Juice or Cider (mg. P/100 ml.)		
		Total Phosphorus	Phosphorus in the Compounds in Solution	Phosphorus in the Material in Suspension
0	1,051	5.75	5.50	0.25
2	1,051½	5.60	5.40	0.20
4	1,051	5.25	5.10	0.15
6	1,049	5.05	4.75	0.30
8	1,046	4.80	4.35	0.45
10	1,043	4.65	3.60	1.05
15	1,027½	4.50	2.35	2.15
17	1,019½	4.20	2.10	2.10
18	1,017½	3.90	2.05	1.85
21	1,012	3.50	2.10	1.40
23	1,009	3.25	2.10	1.15
24	1,008	3.10	2.20	0.90
27	1,005½	2.85	2.35	0.50
30	1,004	2.85	2.50	0.35
34	1,003	3.00	2.80	0.20
41	1,003	3.20	3.00	0.20
55	1,003	3.45	3.35	0.10
76	1,003	3.65	3.60	0.05
104	1,003	3.80	3.75	0.05
132	1,003	3.75	3.70	0.05

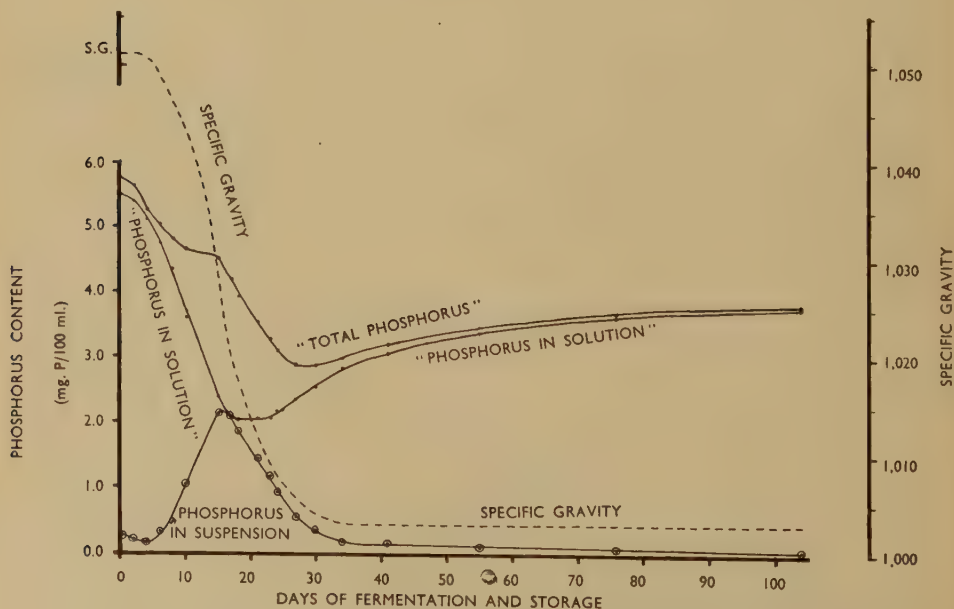


Fig. 3. Changes in the Phosphorus Content of the Juice in Cask I during Fermentation and Storage.

(a) *Phosphorus in the Compounds in Solution.*

"Soluble phosphorus" (Fig. 3) decreased markedly during the first four days and although only a slight decrease occurred during the first two days, there was no definite lag-period such as was observed for "soluble nitrogen." Between the 6th and 15th days "soluble phosphorus" continued to decrease rapidly (5.10 to 2.35 mg.) corresponding to the decrease which occurred in "soluble nitrogen" during that time. After the 15th day the rate of decrease became slower and the minimum value (2.05 mg.) was reached about the 19th day, i.e. about five days *earlier* than the minimum value for "soluble nitrogen." When "soluble phosphorus" was at its minimum the specific gravity of the cider was 1.016.

"Soluble phosphorus" remained approximately constant between the 19th and 23rd days but after the 23rd day a *marked increase* in "soluble phosphorus" was observed. This increase was relatively much greater than the corresponding increase observed in the "soluble nitrogen" content of the juice. The rate of increase in "soluble phosphorus" was particularly rapid between the 25th and 35th days after which it became progressively slower. The amount of "soluble phosphorus" present in the cider on the 132nd day (24/4/1950) was 3.70 mg., which represented *an increase of 80% of the minimum* "soluble phosphorus" recorded (2.05 mg.). At this time "soluble nitrogen" had increased by only 33.3% of the minimum value.

(b) *"Total Phosphorus."*

During the first four days, "total phosphorus" decreased from 5.75 to 5.25 mg. and although "soluble phosphorus" also decreased it did so to a smaller extent. The difference between the "total phosphorus" and "soluble phosphorus" contents at the end of the four days indicated that sedimentation of "phosphorus in suspension" had occurred during this period. Between the 4th and 10th days "total phosphorus" continued to decrease rapidly but the rate of decrease of "soluble phosphorus" was even more rapid and the amount of "phosphorus in suspension" must therefore have increased.

There was very little decrease in "total phosphorus" between the 10th and 15th days although "soluble phosphorus" decreased markedly during this time. These changes are similar to the corresponding changes observed in the nitrogen content of the juice. After the 15th day the "total phosphorus" content of the juice again decreased rapidly and reached its minimum (2.85 mg.) on or about the 27th day, i.e. approximately seven days before the minimum "total nitrogen" was reached. The *increase* which occurred in "total phosphorus" after the 30th day was due to the increase in the "soluble phosphorus" and was relatively greater than the corresponding increase in "total nitrogen."

(c) *Phosphorus in the Material in Suspension.*

The form of the graph (Fig. 3) showing the changes which occurred in the amount of "phosphorus in suspension" during fermentation is almost identical with that of the graph (Fig. 2) showing the corresponding changes in "nitrogen in suspension."

After an initial *decrease* during the first four days, the amount of "phosphorus in suspension" *increased* rapidly and the peak value (2.15 mg.) was reached between the 15th and 16th days corresponding to the peak value for "nitrogen in suspension" which occurred at the same time. The *decrease* in "phosphorus in suspension" after the 16th day was rapid and the *rate* of decrease was constant up to the 27th day. At this time the rate of decrease

became progressively slower and on the 34th day the amount of "phosphorus in suspension" was very small (0.20 mg.); during the next three months it became insignificant in amount.

(4) Autolysis.

When autolysis of yeast takes place, compounds containing nitrogen and/or phosphorus are liberated. The increases which occurred in the "soluble nitrogen" and in the "soluble phosphorus" contents of the cider under investigation were presumably due to the formation of such autolytic products. The exact time when autolysis commenced is not known and autolysis is here considered in relation to the increase *observed* in "soluble nitrogen" and in "soluble phosphorous" shortly after these "constituents" of the cider had reached their minimum values.

There was a relatively large increase in the "soluble nitrogen" content of the cider and this took place during three and a half months after the 27th day of fermentation, at which time "soluble nitrogen" was at its minimum, 8.7 mg. (i.e. 8.7 mg. N/100 ml.). This increase (2.9 mg.) is equivalent (a) to 15% of the nitrogen content (19.4 mg.) of all the yeast which was present at the beginning of this period and (b) to 17% of all the "soluble nitrogen" which was utilised (17.1 mg.) by the yeasts during the major part of the fermentation, (i.e. during the first twenty-seven days). In the *previous* investigation, the corresponding increase in "soluble nitrogen" was of the order of 11% of the nitrogen content of the yeast present and was equivalent to approximately 13% of all the "soluble nitrogen" utilised by the yeasts during the major part of that fermentation.

The method of calculating the nitrogen content of the total amount of yeast which was present (in suspension and in the lees) at any time during the fermentation has previously been described (1). It should be borne in mind that the total amount of yeast *present* at any time includes the yeast which was *originally* present in the fresh juice and that which has grown up to that time.

The greater part (more than half) of this autolysis took place in the first month of the period of three and a half months just considered and the rate of autolysis was most rapid during the first ten days of this month, when there was an appreciable amount of yeast in suspension. This does not necessarily mean that yeast in suspension was autolysed. An alternative explanation is that the slow fermentation, which was still proceeding at that time, caused some disturbance of the yeast in the lees, thus facilitating its autolysis and also distributing the autolytic products of the yeast throughout the volume of cider in the cask.

The "soluble phosphorus" content of the cider will now be considered in relation to autolysis. There was a very marked increase in "soluble phosphorus" (relatively much greater than in "soluble nitrogen") and this took place during the three and a half months after the 19th day of fermentation, at which time "soluble phosphorus" was at its minimum, 2.05 mg. (i.e. 2.05 mg. P/100 ml.). This increase, (1.65 mg.) is equivalent (a) to 45% of the phosphorus content (3.70 mg.) of all the yeast which was present at the beginning of this period and (b) to 48% of all the "soluble phosphorus" which was utilised (3.45 mg.) by the yeast during a major part of the fermentation (i.e. during the first nineteen days).

The method of calculating the phosphorus content of the total amount of yeast which was present (in suspension and in the lees) at any time during the

fermentation is similar to that previously described for nitrogen (1) i.e. it is represented by the difference between the "total phosphorus" *originally* present in the fresh juice and the "soluble phosphorus" present at that time.

It is of interest to compare the relative amounts of "soluble nitrogen" and "soluble phosphorus" which were liberated into the cider as a result of autolysis, with the relative amounts in which these two elements were *present* in the yeast prior to autolysis. Thus, the proportion (45%) of the phosphorus content of the yeast which was liberated in a soluble form as the result of autolysis was three times as great as the proportion (15%) of the nitrogen content which was liberated. This may be explained by the fact that not all the products of autolysis are soluble and that the proportion of the liberated phosphorus compounds which was soluble may have been greater than the corresponding proportion of the liberated nitrogenous compounds.

DISCUSSION.

This further study of the changes occurring in the nitrogen content of apple juice and cider during fermentation has yielded results which are in general agreement with those of the previous investigation (1). There are, however, certain important differences and it is of interest to compare the two fermentations since, although both were fermentations of a Bramley juice, the juice in pipe F (1) was of relatively low nitrogen content ("soluble nitrogen" 7.7 mg. N/100 ml.) compared with that in cask 1 (25.8 mg. N/100 ml.) of this investigation. Furthermore, the changes which occurred in the phosphorus content of this juice during fermentation have also been studied and the relation between these changes and the corresponding changes in the nitrogen content are of fundamental interest. It is not possible to discuss this relation in detail but attention must be drawn to the striking similarity between the changes in the phosphorus contents ("soluble phosphorus," "total phosphorus" and "phosphorus in suspension") of the juice during fermentation and the associated changes in the corresponding nitrogen contents.

This Bramley juice (cask 1) had a specific gravity of 1.051 and fermented rapidly, as expected from its high nitrogen content. The rate of fermentation (over the range from the original gravity down to S.G. 1.008) was approximately one and a half times as great as that of the fermenting juice in pipe F (1). A comparison of the two fermentations will now be made with respect to (i) the amount of nitrogen utilised by the yeasts (i.e. the amount by which the "soluble nitrogen" originally present in the fresh juice decreased up to the time minimum "soluble nitrogen" was recorded), (ii) the period of time during which the utilisation took place and (iii) the amount of sugar fermented (as represented by the decrease in specific gravity which occurred).

Thus in cask 1, the amount of "soluble nitrogen" utilised during the first twenty-four days of fermentation was 17.1 mg. and the loss in specific gravity was 43 degrees. Each of these "changes" is approximately three times as great as the corresponding change in pipe F in which 6.2 mg. of "soluble nitrogen" was utilised in eight days and the loss in specific gravity was 15 degrees. It follows therefore that in these two fermentations (a) the overall amount of yeast which grew in the juice in cask 1 was approximately three times as great as that which grew in pipe F., (b) the relative times during which yeast growth occurred were also in the ratio 3:1, i.e. the amounts of "soluble nitrogen" utilised per day or per 10 degree loss in specific gravity (representative of yeast growth per day or per 10 degree loss in specific gravity) (Ref. 9) were approximately the same and (c) the relative amounts of sugar

fermented were also in the ratio 3:1. In these two fermentations, therefore, the amounts of yeast which grew could be directly related to (in fact were directly proportional to) the respective amounts of sugar fermented and the average rate at which the sugar was fermented during this period of yeast growth was approximately the same in each fermentation. These facts are of considerable interest since the two Bramley juices differed widely in their nitrogen contents but had sugar contents of the same order. In these two fermentations therefore, which took place over approximately the same temperature range, it would appear that the *average* rate of fermentation during the *period of active growth of yeast* was dependent upon the *concentration of sugar* in the juice rather than upon its nitrogen content but other factors are also involved. It should be emphasised that the above comparisons are made with respect to that period of fermentation during which yeast was actively growing and not to fermentation in its entirety. In later work an attempt will be made to relate the different rates at which fermentation proceeds during the fermentation as a whole, with the growth phases of the yeast.

Utilisation of "soluble nitrogen" as discussed above implies a knowledge of the "soluble nitrogen" which was originally present in the fresh juice and the "minimum" to which it decreased as the result of growth of yeast. Shortly after "soluble nitrogen" reached its *recorded* minimum, an increase in "soluble nitrogen", presumably due to autolysis of yeast, was observed. These two processes, utilisation of nitrogen, i.e. removal of nitrogen from solution, and liberation of nitrogen into the cider, are therefore presumably operating at the same time and are opposite in effect. The exact time at which autolysis commenced is, however, not known and it is possible that it started *before* "soluble nitrogen" reached its recorded minimum. If this was so then the value to which "soluble nitrogen" did in fact decrease (as a result of its utilisation for yeast growth) would be masked by the "soluble nitrogen" which was being liberated into the cider (as a result of autolysis) and the minimum value recorded for "soluble nitrogen" would be rather higher than the true minimum. It follows also that the time at which the true minimum "soluble nitrogen" was reached, i.e. the time at which utilisation of nitrogen by the yeast ceased, cannot be precisely determined.

The fraction of the "soluble nitrogen" originally present in the fresh juice which was utilised for yeast growth was approximately 66%, but it follows from the above reasoning that the fraction *actually* utilised may have been slightly greater. The corresponding fraction for the fermenting juice in pipe F was approximately 80%.

It is convenient at this stage to consider the utilisation of *phosphorus* during the fermentation. The course of utilisation of "soluble phosphorus" during fermentation was closely similar to that of "soluble nitrogen." During the period of rapid yeast growth (6th to 15th days) the amount of "soluble phosphorus" utilised was 2.4 mg. (i.e. 2.4 mg. P/100 ml.) and during this time 12.4 mg. of "soluble nitrogen" (i.e. 12.4 mg. N/100 ml.) was utilised, i.e. the relative amounts of nitrogen and phosphorus utilised were in the ratio 5.2:1. "Soluble phosphorus" decreased to its observed minimum approximately five days *before* the minimum "soluble nitrogen" was recorded.

The *material in suspension* in the cider will now be considered in relation to its nitrogen and phosphorus contents. "Nitrogen in suspension" increased rapidly and attained its peak value a few days (approx. nine days) before "soluble nitrogen" had reached its minimum. The amount of "nitrogen in suspension" when at its maximum was 10.7 mg. and is representative of

the yeast in suspension. At this time the amount of "nitrogen in the lees" was only 6.7 mg., i.e. a considerable proportion of the yeast which had grown was in suspension, and the ratio of the yeast which was in suspension to that which was in the lees was 5:3. This ratio was much greater than the corresponding ratio (1:3) for the fermenting juice in pipe F. The amount of "nitrogen in suspension", when at its peak, in cask 1 was approximately six times as great as that in pipe F and it is of interest that such great differences can occur.

Considering now the phosphorus content of the material in suspension, the changes observed in "phosphorus in suspension" were strikingly similar to those observed for "nitrogen in suspension." The peak value of "phosphorus in suspension" (2.15 mg. P.) was reached at the same time as that of "nitrogen in suspension" (10.7 mg. N.); the amount of "nitrogen in suspension" when at its peak was therefore five times as great as the amount of "phosphorus in suspension" at that time. The nitrogen/phosphorus (N/P) ratio of the material in suspension at other times, both before and after the peak, was also approximately 5:1. Such constancy in the N/P ratio would be expected if the nitrogen and phosphorus were present in a single material. The material in suspension was yeast and it follows that these two elements, nitrogen and phosphorus, were present in the yeast in the ratio of 5:1.

The changes which occurred in the composition of the cider as the result of autolysis of yeast have already been described in the section on autolysis. The increase (1.65 mg.) which occurred in the "soluble phosphorus" content of the cider was relatively greater (i.e. with regard to the amounts of these two elements *present* in the yeast) than the corresponding increase (2.9 mg.) in "soluble nitrogen" and the ratio of the amounts of nitrogen and phosphorus thus liberated into the cider was only 1.8:1 (approximately). This ratio is quite different from the ratio in which these two elements were *present* in the yeast i.e. 5.0:1 (as calculated when yeast in suspension was at its maximum) and also from the ratio in which the two elements were *utilised* by the yeast for growth i.e. 5.2:1. It would appear therefore, that relatively more phosphorus than nitrogen (as compared with the amounts of these two elements present in the yeast) was liberated in soluble form from the yeast as a result of autolysis.

The observations on the changes in the sugar content of the juice during fermentation require little comment. It is of interest that sucrose was hydrolysed at a rate such that the reducing sugar content of the fermenting juice remained constant during the first ten days of fermentation, although during this time approximately 2 g. sugar/100 ml. were fermented. The significance of this will be discussed in a later paper.

Knowledge of the manner in which yeast is distributed throughout a fermenting juice, i.e. in the lees and in suspension, is of practical importance in cider-making. For example, the process of racking, which is frequently carried out to check fermentation, will not remove yeast in suspension but only that in the lees. The relative efficacy of yeast in suspension and of that in the lees in bringing about fermentation is not yet fully understood but it can be stated that racking would be more effective if carried out some time after yeast in suspension has reached its peak, preferably at a time when it has decreased to a relatively low value. On the other hand, centrifuging will remove practically all the yeast, both that in suspension and that in the lees, and it is the amount of residual "soluble nitrogen" which largely determines the stability of the cider. Filtration, through pulp, filter-sheet or kieselguhr, is usually more efficient than centrifuging in effecting the removal of yeasts and the stability of the resulting cider will again depend upon the amount of

"soluble nitrogen" remaining. As stated in a previous paper (2) it is possible that the stability of a centrifuged cider is dependent upon factors other than the "soluble nitrogen" content of the cider, and this would apply also to cider which has been filtered.

An indication has been given above of the manner in which the results of this study of the changes which occur in the nitrogen and phosphorus contents of a juice during fermentation may be applied to the practice of cider-making. The factors which determine the course of fermentation and the stability of the resulting cider are not completely understood. Certain directions in which further studies should proceed have been indicated earlier in the discussion.

SUMMARY.

(1) The fermentation of a Bramley juice has been further studied with particular reference to the changes in the nitrogen content of the fermenting juice and the associated changes in specific gravity; observations on the sugar content of the juice during fermentation have also been made.

(2) Comparisons of the nitrogen changes which occurred during this fermentation and those in the fermentation previously described (1) have been made.

(3) Changes which occurred in the phosphorus content of the juice during fermentation have been characterised.

(4) The course of the utilisation of both "soluble nitrogen" and "soluble phosphorus" by the yeasts during the fermentation has been studied. Approximately 66% of the "soluble nitrogen" and 63% of the "soluble phosphorus" originally present in the juice were utilised during the fermentation.

(5) The ratio of the amount of yeast in suspension, when at its maximum, to that in the lees at that time was 5:3. In the fermentation previously described the corresponding ratio was only 1:3.

(6) When yeast in suspension was at its maximum the ratio in which nitrogen and phosphorus were present in the yeast was 5:1.

(7) Observations on the autolysis of yeast which occurred during fermentation and storage have been made. The proportion of the phosphorus content of the yeast which was liberated into solution as a result of autolysis was three times as great as the corresponding proportion of nitrogen.

(8) An indication is given of the manner in which the results of this work may be applied to the processing of a fermenting juice in cider-making.

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A PRELIMINARY TRIAL OF YEASTS FOR THE DOMINANT FERMENTATION OF CIDERS.

By B. T. P. BARKER.

INTRODUCTION.

Since research on cider-making began at Butleigh Court in 1893 on the initiative of the Bath & West and Southern Counties Agricultural Society, and after its transference to Long Ashton on the establishment of the National Fruit and Cider Institute, the control of the character of the fermentation of the liquor by the use of selected yeasts has been the subject of investigation from time to time. (1), (2), (3), (4), (5), (6). The earlier experimental work was carried out under conditions which permitted little more to be achieved than to demonstrate that the yeasts used for this purpose produced ciders with somewhat distinctive aromas, flavours and other characters dependent upon the individual yeasts themselves. Lack of staff and necessary equipment prevented the work from being carried much beyond that stage at that time.

With the increase in personnel and general facilities at Long Ashton since the end of the last war it has become possible to revive research on this subject in a more detailed manner. The ultimate practical aims concerned are to find the most suitable yeast or yeasts for cider production and the most appropriate technique for their use under English conditions to permit of controlled and standardised fermentations.

The nature of the work associated with the more detailed studies rules out of account the simultaneous large-scale trial of more than a few yeasts in any single year.

The selection of a promising group of a small number of yeasts for trial has been necessary therefore for the new investigations. Accordingly, with this object in view, the preliminary tests described in this paper were carried out during the cider-making season 1948-9. A fairly extensive collection of yeasts has been maintained at the Research Station since its establishment and during recent years several additions to it have been obtained from various sources. It is from this collection that the yeasts utilised in the preliminary tests have been drawn.

The chosen yeasts must be satisfactory in respect of the following characters:—

1. *Aroma and Flavour.* Fermentation as regards aroma and flavour effects must yield a product either with a distinctive and attractive character resulting from the fermentation or, alternatively, one with relatively negative character which permits the initial fruit "apple" character of the unfermented juice to persist in the final product.

2. *Body and Smoothness.* Body is difficult to define precisely, but very definite to the palate. The degree of body desirable varies according to the precise type of drink required. For a light drink full body tends to detract from its "light" character, but sufficient is necessary to avoid any suggestion of "thinness" or "watery" nature. A full-bodied drink on the contrary, as its name implies, calls for the character in substantial and unmistakeable form.

Body is undoubtedly closely associated with the raw material from which the drink is made. Nevertheless there is also an apparent yeast effect: for

when the same liquor is fermented by two different yeasts the product in the one case may be distinctly "lighter" in body than in the other.

Some yeasts can give harshness, roughness or coarseness to a full-bodied cider: with a "light" drink the effect is one of rawness. Others produce a soft smoothness or "silkeness" which adds greatly to the attractive character.

3. *Vigour and Attenuation Power.* Growth vigour under the nutritional conditions of apple juice is needed for dominant fermentation, the "culture" yeast having to suppress as far as possible the competition of the naturally occurring wild yeasts.

Attenuation power is distinct from growth vigour and not always associated with it. Where vigorous fermentation is desired, high attenuation power is valuable—especially for apple juices which so frequently are deficient in assimilable nitrogen. Competition for the latter between the "culture" yeast and the wild yeasts is keen and so, to ensure that the specific gravity of a given juice can be reduced to any desired point within a reasonable space of time and a satisfactory yield of alcohol obtained from the sugar fermented, the combination of growth vigour and attenuation power in the selected yeast is requisite.

Where the objective is a naturally sweet cider of relatively high gravity other considerations arise. While, for example, present-day methods of arresting fermentation by centrifuging and filtration are very efficient, it is not always practicable during rush periods to apply the necessary processing in time. In such cases a "culture" yeast of high growth-vigour but low fermentation and attenuation qualities might prove helpful in deferring the date for processing. It might even be still more effective to use also in combination an additional strong-growing non-fermenting yeast.

Thus with suitable yeasts there is very considerable scope for fermentation control in cider-making owing to the normal relatively low amount of assimilable nitrogen in apple juices.

4. *Clarity.* One of the essentials in present-day commercial ciders is a very high degree of clarity. However effective the artificial clarification by filtration may have been, a cider is not necessarily rendered yeast-free and more or less yeast growth may occur after some lapse of time: consequently the liquor may become turbid to some degree or a deposit may settle on the base of the container. Such deposit may be firm in texture and then the liquor can be poured from the bottle without loss of clarity: at other times the deposit may be loose or flocculent, in which case it is difficult to prevent its disturbance and consequently clouding of the liquor on decanting. In these respects individual kinds of yeasts differ considerably in their behaviour.

Yeasts giving good clarity also can render the clarification processing—racking, centrifuging and filtration—relatively speedy and easy.

EXPERIMENTAL.

The objective of the preliminary trials here described was the selection of a group of 5–10 yeasts of sufficient and varied character for large-scale trials to illustrate what could be achieved by dominant or pure culture fermentation in improvement on the results of the usual chance spontaneous fermentation.

The procedure adopted was similar to that used on various previous occasions when practical considerations had made it impossible to try out more than a very limited number in the final examination.

In such cases small-scale laboratory trials were conducted in small vessels—flasks or bottles—containing about 500–1000 ml. of the test liquor. The liquor after sterilisation in the containers plugged with cotton wool was inoculated

with active pure cultures of the yeasts under trial and the resulting fermentation was allowed to proceed until it reached completion. The appropriate observations were then made and the results in the individual cases compared. The ultimate choices for the major large scale trial were then made.

The trials in the present case were carried out in champagne pint bottles. Each yeast was tested in a Sweet Alford juice. In addition supplementary series of tests were made in three other juices—Morgan's Sweet and two miscellaneous blends. It was not possible in the time available to include every yeast in each of the supplementary tests, but the trials were so arranged that each yeast was included in one or other of the latter.

The number of yeasts examined in these tests exceeded 75. Their sources of origin have been highly varied. The largest group consisted of yeasts isolated over a long period of years at Long Ashton from freshly expressed apple juices and ciders at various stages of fermentation: they include several yeasts obtained from Kingston Black ciders. In most instances the ciders concerned were made at the Research Station, the remainder coming from makers in other districts. Two different cultures of cider distillery yeasts were also included, as well as two types (Fendant and Herrliberg) widely used in Switzerland for cider fermentation in that country.

A substantial number of wine yeasts obtained from different sources constituted another group. The various types as named by the suppliers were as follows:—Arak, Champagne Hauteville and another champagne yeast, two Californian wine yeasts, Dortmund, Douro Laureto (a port yeast), two Hungarian wine yeasts, Nos. 4536 and 4537, Jeres (a sherry yeast), Johannisberg, Laclairé, Moschatel, Sauterne, Steinberg, Tokay, Wageningen I and II, Winningen, and Zeltingen.

Isolates from various English fruit juices—particularly blackcurrant—numbered ten. Two further samples came from fermenting orange juice. Four cultures originated from dried Australian grapes. Another was isolated from an old-style fermented ginger beer and was derived originally from a piece of ginger rhizome.

RESULTS.

After inoculation the respective juices were kept under observation at ordinary laboratory temperatures. It was soon evident that they sorted themselves into well-defined groups. One group—much the largest numerically—showed clear signs of incipient fermentation within a few days. The other, comprising only six samples, gave little indication of fermentation in the first week and at no time in later life did it become vigorous in any instance: in two cases the specific gravity remained stationary at only a few points below the initial gravity.

Throughout the active fermentation period which followed—except in the two cases just mentioned—there was little of special interest to record. As it drew to its close, visible signs of difference in degree and rate of clarification were noted, and a few weeks later, when fermentation had ended—except for some of the group two samples—fairly clear-cut differences in clarity were apparent. Few could be described as clear without haze; the majority showed haze in various degrees, although considerable clearing had occurred: some were very definitely cloudy and in a few instances the condition could best be described as “muddy.”

With no obvious material changes taking place after another interval of some weeks the samples were examined for flavour, gravity and clarity. The

results of this examination are given in summarised form and attention called to any individual cases showing noteworthy features.

Aroma and Flavour.

The fact that all samples were autoclaved at the outset of the trials, to ensure complete sterility before infection with the yeasts, involved radical changes in aroma and flavour of the juice. Thus those characters cannot be assessed on the same basis as those of unsterilised material. However, comparison with an unfermented sterilised sample retained for the purpose served to show to what extent and in what manner the flavour and aroma had been modified by the action of the individual yeasts. The characters exhibiting substantial differences were (a) acidity level, and (b) flavour quality in respect of degrees of softness, coarseness and fullness.

The aroma naturally had a marked "cooked" character before infection. The yeast effects on this differed considerably in individual cases and for the most part varied between "little change" and a marked yeasty aroma, usually unpleasant in extreme cases, but at times not unattractive and in a few instances suggestive of volatile esters. One example was outstanding in that respect: the yeast concerned was consequently placed on the list of those selected for the 1949 large scale trials in the ciderhouse.

The difference in degree of sharpness in flavour throughout the series was very considerable. The apple juice under test was "Sweet Alford," a member of the "sweet," "low-acid" class, specially selected as suitable to show clearly "acidity" variations between the trial yeasts. Its original acidity was 0.12 per cent (expressed as malic acid) and the pH 4.2. The pH range after the fermentations was from less than 3.6 to a high level of over 4.5. In the majority of cases the level was from 3.9 to slightly above 4.2: within these figures the flavour was not unduly sharp nor flat, but outside these limits the flavour became unbalanced through the pronounced acidity or flatness in the respective cases.

Body and Smoothness.

Some of the ciders possessed an exceptional degree of softness and a fair number showed that character in reduced form. Many, on the other hand, had developed a harsh rough character which rendered them unpalatable. A substantial number were noted as clean in flavour and had evidently not suffered through the unavoidable long standing of the liquor on the lees, with the attendant risk of yeasty flavour taints due to autolysis of some of the yeast cells.

There were marked differences in fullness of flavour and body. Generally fullness in those respects suited the type of juice used in the trials: with a richer type of juice, such as one of the "fuller" bittersweet varieties, it is possible that the fullness mentioned might prove too much and yield too "heavy" a product. There were no outstanding examples of deficiency in body or flavour fullness; the juices selected for the trials had sufficient character in those respects to obscure any yeast deficiencies of that nature.

Vigour and Attenuation Power.

As the visual observations on the times of onset of active fermentation already noted have suggested, some outstanding cases of extreme difference in attenuation had occurred. The ultimate specific gravity readings were taken when the ciders were approximately ten months old.

The greatest attenuation was found in five instances, the gravity being 1001. Another five ciders showed gravities of 1002, while the majority of the remainder ranged between 1003 and 1005. Eighteen were placed in the gravity range 1006–1008. The few above that level included one at 1048, two at 1047, one at 1039, one at 1018 and one at 1015 : of these, the two latter were fermented by yeasts obtained from orange juices and the remainder by yeasts isolated from ciders.

The control cider, made by inoculating a bottle of the pasteurised Sweet Alford juice with a few ml. of the same juice unpasteurised, gave a gravity of 1013. In this instance, since the inoculum included not only the yeasts naturally present in the juice but also acetic and other bacteria, acetification began as the vigour of the alcoholic fermentation subsided and the gravity figure recorded probably is a fair indication of the degree of attenuation by the yeasts of natural occurrence up to the point where acetification brought further yeast action to practically a standstill.

Clarity.

Observations on the clarity of the individual samples at the end of the active fermentation period have already been mentioned. After a further year's storage there has been little change : such as has occurred has tended to be in direction of decrease rather than increase.

Very few could be described as clear, and only one or two as brilliant. Most had cleared to a point of clarity where newsprint was fairly legible through a layer of the liquor about an inch in depth : nevertheless when in the glass, a very marked haze of different shades of density was the feature which caught the eye.

To some extent it is probable that these results were due partly to the kind of juice used in the trials and not entirely to the exclusive action of the individual yeasts. Low-acid juices of the Sweet Alford type—as used here—tend to develop a very persistent type of haze on pasteurisation at temperatures of 100°C: sometimes after standing for a long period the liquor clears and a solid film is deposited on the sides and bottom of the container, but the clarification is frequently more or less incomplete.

DISCUSSION.

Reviewing these results it is evident that there should be no difficulty in finding yeasts able to attenuate juices to the extreme limit possible. Whether such yeasts, when tested for dominant fermentation purposes under practical conditions and given all possible material aid, can achieve the necessary degree of dominance over the naturally occurring micro-organisms in apple juice is a matter on which the 1949–50 trials should provide some answer.

Even if dominance is satisfactorily achieved, the flavour question may be complex. It would appear from the results obtained by Continental workers that juices of different composition do not necessarily respond in the same way to a given yeast : the flavour in one case may be quite satisfactory, while in a different juice the result may be very disappointing (Charley, 7). Thus, except with juices of reasonably constant composition, a selected yeast cannot apparently be relied upon to give standard results in flavour. As the cider fruit situation stands at the present time, standardisation of juice composition in general is out of question. While that position prevails, the most promising line of development seems to be in the direction of special yeasts for special types of juice. The trials described in this paper show that from the yeasts

tested degrees of acidity, fullness of flavour and body and softness on the palate can be provided in considerable variety.

With present day facilities for artificial clarification the efficiency of a yeast in respect of the production of a product of a high grade of clarity has become rather a secondary matter. This point of yeast behaviour cannot be left out of account entirely; for, except with pasteurised ciders, yeast growth does take place to some degree after final clarification in most cases. Hence it is desirable that a selected yeast should possess the property of yielding a firm deposit.

The group of yeasts with low powers of attenuation should be kept in mind, should further research on fermentation rates of juices by mixtures of yeasts of different attenuation values show that appreciable rate variation can be achieved. Theoretically such a result might well occur, since in a fair number of freshly-pressed juices assimilable nitrogen is too low in amount to give maximum fermentation rates or even complete attenuation: under such conditions if two or more yeasts of widely differing attenuation powers are present at the outset in fairly equal numbers, with each assimilating its share of the available nitrogen for cell growth and multiplication, the most active "attenuator" might be expected to suffer correspondingly.

If juice pasteurisation were to become a regular practice, or if other means of producing a sterile juice emerge, the low-attenuating yeasts then might repay closer attention for use in the making of sweet cider.

With these considerations in mind a group of eight yeasts has been selected from these trials for the large-scale trials on juices from the 1949 apple crop. A detailed account of the latter will be given in the next Report.

SUMMARY.

1. With a view of selecting a small group of yeasts for use in dominant fermentation trials on a cider-house scale on juices from the 1949 apple crop small-scale preliminary tests with over 75 different yeasts were made with juices from the 1948 crop.

2. Observations on these were continued over a period of ten months. In each case the following points were noted:—aroma, flavour, vigour of fermentation and extent of attenuation, final pH and degree of clarity.

3. The more important features of each of these characters in relation to cider production are discussed. Taking them into account, a selection of eight yeasts from the group on trial has been made for the further work in progress.

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AN INVESTIGATION ON THE YEAST FLORA OF KINGSTON BLACK CIDERS.

PART I

By B. T. P. BARKER.

INTRODUCTION.

Before the establishment of the National Fruit and Cider Institute late in 1903 little was known about the yeasts responsible for the fermentation of English ciders. In actual cidermaking practice the apple juice, after expression, was allowed to ferment spontaneously, this being brought about by the wild yeasts which chanced to be present at that stage. It was known that yeast cells normally were to be found in varying numbers on the surfaces of sound apples and more numerous in the exposed flesh tissues of the fruit after skin abrasions, especially where bruises and fungal invasions had caused the onset of rotting in the internal tissues. In the course of milling and pressing operations many of these passed into the freshly expressed juices. Other possible sources of juice infection were the mills, presses and other items of cidermaking equipment with which the liquor came into contact before reaching the fermentation vessel itself, as well as the latter if not previously sterilised. Thus the microflora of the juice at the outset was inevitably mixed in character and origin. F. J. Lloyd, in the course of his investigations during the period 1894-1903, made a few pure-culture isolations of yeasts from various juices and ciders and in some cases used them for pure-culture or dominant-fermentation trials on selected juices. (1). No detailed descriptions of the characters of the respective yeasts were published by him : hence it is impossible to identify them.

During the earliest years following the establishment of the Institute a considerable collection of yeasts isolated from apples and ciders was accumulated and some knowledge of their characters gained. This sufficed to show that from such sources an indefinitely large number of distinct types of varying degrees of interest could be collected with very little effort. That being the case, the available detailed characters of the individual forms of so miscellaneous a collection were not considered to be of sufficient value or interest to justify publication, although the generalised results have been recorded in a paper on the microbiology of cidermaking (2).

From time to time closer attention has been given to specific aspects of the yeast flora, as for example that of bottled ciders (3). In other cases, for various reasons, the results of the work have not been published : the subject of the present paper is concerned with one of these cases. Charley, in his recently published text-book on cider (4), has referred to Lloyd's work on cider yeasts, and quotes that investigator's claim that a yeast isolated by him from Kingston Black apples proved capable of giving a " Kingston Black " character to ciders made from juices of other varieties of apples when used for their fermentation. Since that work was largely responsible for the initiation of the investigation here described, it seems desirable that the results of the latter should now be placed on record.

Aroma and Flavour in Relation to Yeast Action.

It has long been accepted that the aroma and flavour of fermented beverages owe their character to some extent to the individual kinds of yeasts concerned

with the fermentations. Thus it has followed that many investigations have been made to find suitable yeasts for the various classes of such drinks, with the view of their use in pure culture form or as dominating organisms to secure products of standard character in those respects as well as others. The degree of success achieved has been very varied. With beer and related liquors, which start with a sterile wort, pure culture fermentation is possible and the selection of a suitable yeast becomes relatively simple. With products such as wine and cider the initial liquor cannot be sterilised without some impairment of the flavour of the fresh fruit juice; consequently the selected cultivated yeast must compete with the "wild" yeasts and bacteria invariably present in the freshly-expressed juice and be able to keep them in suppression sufficiently to impose its own character in the fermentation.

In the case of cider it has been suggested from time to time that certain varieties of apples, e.g. Kingston Black, noted for the characteristic flavour and aroma of the ciders made from their juices, owed those features—in part at least—to the presence of one or more distinctive yeasts associated with their fruit and thus of normal occurrence in their freshly-expressed juices. As previously mentioned, as far back as 1894 F. J. Lloyd reported (5) that, when using a yeast derived from that source for fermenting the juice of other apples, he had succeeded in producing a cider bearing, to a certain extent, the true Kingston Black character.

Such views and results imply the existence of certain yeasts capable of producing the typical features of the ciders of individual varieties of apples of highly distinctive vintage character, such as Kingston Black. A further implication is the normal association of such a yeast flora—either in the form of a single variety or a group of varieties—with the juices of the apple in question. Hence the use of the descriptive name "Kingston Black" yeast. These implications hitherto have neither been proved definitely nor disproved.

EXPERIMENTAL.

The Nature and Scope of the Investigation.

The investigation described in this paper was undertaken to obtain definite evidence on that subject. Since, as regards English ciders, those made solely from the Kingston Black apple are generally accepted as normally being outstanding in vintage quality and possessing a distinctive character of aroma and flavour, it was decided to make a detailed examination of their yeast microflora. An additional point influencing this selection was that at that time, Kingston Black was the only variety of cider apple common to each of the cidermaking counties of the West of England: thus it afforded the opportunity of securing samples of fruit grown in a widely scattered range of districts and on varied soils. Under such conditions, if the results showed that one or more kinds of yeast were common to all the samples examined and were not of normal occurrence in ciders made from other apples, there would be a strong case for examining closely their behaviour when used to ferment juices of other varieties. Should these other juices, after fermentation, then exhibit the characteristic "Kingston Black" aroma and flavour, the existence of a "Kingston Black" yeast or yeasts might be considered as established.

An investigation along these lines was begun in 1908. In the autumn of that year half-ton consignments of Kingston Black apples were obtained from each of the following centres:—Budlake (Devon), Corse Lawn (Hereford), Edgborough (Somerset), Martock (Somerset), Pendock (Worcester), and Staplegrave (Somerset). The fruit, on arrival at Long Ashton, was stored

under cover in a well-ventilated loft until it reached a suitable stage of maturity for expression of the juice. The precise period of storage differed in individual cases.

The freshly expressed juice in each case was immediately placed in a clean hogshead, thoroughly steamed just before use to kill any actively-growing yeast cells and bacteria which might have been present. Fermentation of the juice was allowed to develop naturally. It was intended originally to allow it to continue unchecked, until the specific gravity had fallen to approximately 1025, at which point it was to be filtered prior to bottling. The fermentations, however, proved to be exceptionally slow; so, to avoid risks of acetification and possible sickness, as well as to ensure some degree of natural conditioning in bottle, the ciders were filtered at gravities of 1035 upwards.

In order to get a comprehensive survey of the kinds of yeasts present throughout the whole course of the fermentation period, it was decided to make a detailed examination of the microflora at three stages. The first examination of each juice (November 25th and 28th, 1908) took place seven days after the date of its expression; at that point active fermentation had not developed, although active multiplication of the yeasts present in the freshly-expressed juice had occurred to a sufficient extent to give incipient fermentation. The second examination (December 21st, 1908) was timed to show the nature of the microflora at the height of primary fermentation. The third examination (October 1909) was deferred until after the fermenting liquor had been clarified by filtration and had been stored in bottles, under the normal conditions for bottled cider, sufficiently long to mature and develop natural conditioning by the slow growth of a "secondary" yeast crop. Apart from any chance infections this "secondary" crop was derived from the residual yeast cells still remaining in the cider after passage through the filter. The bottling was done direct from the filter. The microflora in this instance consisted of the micro-organisms concerned with maturity changes of aroma and flavour directly due to microbiological action.

Isolation of the individual yeasts in all cases was accomplished by the fractional plate-culture method. The samples were taken direct from the main bulk of the liquor in cask by sterile pipettes in the first two examinations; in the third examination the samples were taken similarly from the bottled product. Beerwort gelatine was used as the medium for the plate cultures, which were kept at ordinary laboratory temperature until the yeast colonies which developed had grown to a size sufficiently large to show distinctive characters in appearance under examination by hand-lens or naked eye. While these macroscopic characters served to effect a preliminary sorting of the different yeast types present in the cultures, the final selection for further culture in each case was not decided until a microscopic examination of the vegetation had been made. This test was applied to several colonies showing corresponding macroscopic appearance: where the cell forms of the vegetation showed differences, notwithstanding the similarity of macroscopic appearance of the colonies, separate isolations were made. Thus, although it is possible that a few kinds of yeast may have escaped notice, it is highly improbable that any of those overlooked were present in sufficient numbers in the cider concerned to have had any material effect on the ultimate aroma and flavour of that cider.

The plate cultures also contained a limited number of colonies of bacteria. Little attention was given to them, since the investigation was solely concerned with the yeast flora: but it should be mentioned that they were mainly those of

acetic bacteria and that in no instance was any kind observed which appeared unusual or had not been noticed in previous examinations of cider microflora.

The characters of the six ciders, as observed on sampling the bottled product in May 1909, are given in the following notes taken from the Annual Report of the Station for that year. The notes also include the dates of milling and pressing, the composition of the freshly-expressed juices in respect of acid and tannin contents and specific gravity, their rates of fermentation at 28°C, and the dates of filtration with the specific gravities at those dates. It will be noted that the individual ciders differed somewhat in their general character; but in spite of such variations all were unmistakably Kingston Blacks. Thus, if that distinctive character was in any way associated with microbiological action, the records of the microflora of the individual ciders should provide some positive evidence in that direction.

Corse Lawn (Hereford)—Analysis of fresh juice, November 18th, 1908: specific gravity 1·066, malic acid ·65 per cent., tannin ·186 per cent. Average daily fall in specific gravity at 28°C., ·0021. Filtered January 25th, 1909: specific gravity 1·038. A sweet brisk cider of good flavour and aroma and fair body. Good alone, although rather on the sharp side.

Pendock (Worcester)—Analysis of fresh juice, November 18th, 1908: specific gravity 1·064, malic acid ·62 per cent., tannin ·196 per cent. Average daily fall in specific gravity at 28°C., ·0026. Filtered January 4th, 1909: specific gravity 1·035. A rich, sweet, fruity cider of a nice degree of briskness; flavour and aroma very good. Excellent alone.

Budlake (Devon)—Analysis of fresh juice, November 21st, 1908: specific gravity 1·056, malic acid ·46 per cent., tannin ·116 per cent. Average daily fall in specific gravity at 28°C., ·0016. Filtered January 4th, 1909: specific gravity 1·037. A sweet, rather thin and somewhat insipid cider of slight briskness; aroma not pronounced and flavour very fair. A useful cider, but lacking in the richness and pleasant briskness natural to the variety on many soils.

Martock (Somerset)—Analysis of fresh juice, November 21st, 1908: specific gravity 1·064, malic acid ·52 per cent., tannin ·126 per cent. Average daily fall in specific gravity at 28°C., ·0022. Filtered January 25th, 1909, specific gravity 1·039. A rich, sweet, full-bodied cider of decidedly soft and rather heavy character; flavour and aroma very good, although marred by a slight earthy taint. An excellent cider of the Somerset type, with a moderate degree of briskness. Blending unnecessary.

Edgborough (Somerset)—Analysis of fresh juice, November 21st, 1908: specific gravity 1·070, malic acid ·56 per cent., tannin ·192 per cent. Average daily fall in specific gravity at 28°C., ·0015. Filtered March 16th, 1909: specific gravity 1·043. A very sweet, soft, rich, heavy cider of medium briskness; flavour and aroma excellent. A first-rate cider alone, although rather too much on the heavy side.

Staplegrave (Somerset)—Analysis of fresh juice, November 21st, 1908: specific gravity 1·058, malic acid, ·41 per cent., tannin ·138 per cent. Average daily fall in specific gravity at 28°C., ·0016. Filtered January 25th, 1909: specific gravity 1·037. A sweet, rich, soft, full-bodied cider of fair briskness; flavour and aroma very good and fruity. A fine, rich cider alone.

The Yeast Flora at the Onset of Active Fermentation.

The rest of the present paper is confined to the results of the first section of the complete investigation, and so deals exclusively with the yeasts isolated from the six ciders in the initial stages of the primary fermentation.

The total number of isolations was forty-six, those from the respective ciders being as follows:—Budlake, 8; Corse Lawn, 7; Edgborough, 9; Martock, 8; Pendock, 6; Staplegrave, 8. Each culture was examined separately in respect of the following characters:—(a) appearance of its colonies on beer-wort gelatine plate and streak cultures, (b) the character of the vegetation in those cultures, (c) maximum temperature of growth on beer-wort agar streaks, (d) action on glucose, fructose, sucrose, maltose and lactose as regards fermentation, (e) ability to form spores on potato and carrot cubes and porous porcelain (at ordinary room temperature and 25°C.), as well as in streak cultures on beer-wort gelatine.

These tests indicated that three of the eight Budlake isolates were very closely allied, but probably not identical yeasts, and two of the remaining

five were indistinguishable from each other. Of the Corse Lawn group, there were two pairs of obvious duplicates, while the Edgborough set included one pair of duplicates. Two of the Martock isolates proved to be alike, with another only slightly different. There were no duplicates among the Pendock group, but two of the Staplegrove set were probably alike. Comparing the yeasts from all sets it was found that they could be divided into eleven classes, characterised as follows:—

Spore-forming

- Class 1 Cells medium to large, ovoid and sausage-shaped (young cultures); maximum growth limit about 34°C; spore-bearing cells in most cases very numerous; ferments glucose, fructose, sucrose and maltose.
- Class 2 Cells medium, round-ovoid, very uniform; maximum growth limit 32.5°C; spore formation preceded by cell conjugation; ferments glucose, fructose, sucrose and maltose.

Non-Spore-forming

- Class 3 Cells medium, roundish-ovoid, very uniform; maximum growth below 30°C; ferments glucose, fructose and sucrose.
- Class 4 Cells medium, ovoid, uniform with conspicuous vacuoles; maximum growth limit 32.5°C; ferments glucose and fructose, forming a film on the surface of solutions of the latter.
- Class 5 Cells small to medium, round and ovoid; maximum growth limit 38–41°C; ferments glucose and fructose; liquifies gelatine media.
- Class 6 Cells medium, sausage-shaped and very marked tendency to mycelioid arrangement; maximum growth limit below 30°C; does not ferment any of the sugars tested, but forms a film on fructose solutions.
- Class 7 Cells medium, ovoid, very uniform; maximum growth limit about 42°C; does not ferment any of the tested sugars, but forms a film on maltose and lactose solutions.
- Class 8 Cells small, ovoid, uniform; maximum growth limit 38–40°C; does not ferment any of the tested sugars; liquifies gelatine media.
- Class 9 Cells small, ovoid, very uniform; maximum growth limit 25–27°C; doubtful slight fermentation of glucose and fructose.
- Class 10 Cells small, spherical and ovoid; maximum growth limit 30–32.5°C; does not ferment any of the tested sugars.
- Class 11 Cells small to large, *apiculatus* type; ferments glucose and fructose.

Class 1 is the most numerous and is comprised of isolates from each of the six centres. In all, it includes fourteen cultures. Of these, three, coming respectively from the Budlake, Corse Lawn and Martock centres, appear to be identical. The remainder, although obviously closely allied to the former and to each other in their characters in general, show minor differences, indicating them all to be distinct forms. Thus separated, the class is made up of three cultures from the Budlake group, one from Corse Lawn, two from Edgborough, three from Martock, three from Pendock and two from Staplegrove.

It should be mentioned that a high proportion of the yeasts isolated at Long Ashton about that period, from a wide range of juices and ciders made

from other apples than Kingston Black, would fall also into this class on classification. As a class, then, it must be considered as one of primary importance in connection with English cidermaking in general, at that time.

Class 2 contains only a single form, that being found in the Corse Lawn group, the isolate of which included a duplicate culture of this type. This yeast belongs to the *Zygosaccharomyces* genus, other members of which have been found in ciders on other occasions (3). Conjugating yeasts, in fact, occur frequently in the microflora of ciders.

Class 3 is made up of two members, one from the Budlake group—in which there was its duplicate among the original isolates—and the other from the Edgborough group. These two cultures are extremely close in their various characters and may be identical. In view of their ability to ferment glucose, fructose and sucrose they probably were of some significance in the fermentation of their respective ciders.

Class 4 is also composed of two cultures, one from the Pendock group and the other from the Staplegrove group. Their differences are slight; but, since these are shown both in cell form and appearance of individual colonies on solid media, the two cultures seem to be distinct.

Class 5 comprises four cultures—one each from the Pendock and Edgborough groups and the remaining two from the Staplegrove group. The characters of the class make it a distinctive one and the individual cultures in it are, therefore, probably closely related, though not identical owing to slight differences in cell form. Owing to the ability of its members to ferment glucose and fructose they may be of some significance in the cider concerned.

Class 6, composed of two cultures—one from Corse Lawn and the other from Edgborough—is another distinctive class with its very low maximum growth temperature and a very characteristic streak culture growth. The only apparent difference between the two cultures is a heavy film growth on fructose solutions shown by the Edgborough yeast as compared with a very flocculent growth in the culture fluid by the Corse Lawn yeast.

Class 7 consists of one culture only—from Edgborough. (A duplicate of this was found in the original isolates). Its chief feature of interest is its unusually high maximum growth limit.

Class 8 also consists of a single culture—from Corse Lawn. Were it not for its apparent inability to ferment any of the sugars tested, it would fall unquestionably into Class 5.

Class 9 includes a single culture only—from Martock. While resembling the Class 8 culture in cell form and size and the liquification of gelatine media, it differs widely in maximum growth temperature limit and has shown a slight sign of fermentation of glucose.

Class 10 with one culture only—from Budlake—shows affinities to Classes 8 and 9, but has a distinctive maximum growth limit of slightly over 30°C.

Class 11 is a composite one containing eight cultures and, like Class 1, includes representatives from each of the six centres. Budlake contributes two, Course Lawn one, Edgborough one, Martock one, Pendock one, and Staplegrove two (these may be duplicates). The predominant cell form in all cases is the characteristic *apiculatus* type—a lemon-shaped cell budding at each end—but in one instance a large proportion of the vegetation consists of cells of ovoid shape. In some of the cultures the average cell size is smaller than that of the remainder. Further differences within the Class are the maximum growth temperatures—ranging from a little over 35°C to below 30°C—the character of the edges of the streak cultures on solid media (irregular in

some cases and even in others), and sparse spore formation in two instances. The latter feature places the two cultures concerned outside the true *apiculatus* group, as represented by the type form *Kloeckera apiculata*. Taking such differences into account, only two of the eight cultures appear to be identical, one coming from Edgborough and the other from Pendock. This is an interesting point, since a prominent feature in the yeast microflora of freshly-expressed apple juices as a whole is the prevalence of *apiculatus* forms.

DISCUSSION.

Reviewing the results of this analysis of the yeast microflora of Kingston Black juices from fruit grown at six widely distributed centres in the same year, it is clear, firstly, that one may expect to find in their earliest stages of fermentation at least from about five to eight distinct kinds of yeast in each sample of juice.

It is also evident that in the cases under examination there was no single kind of yeast common to all the six juices. The notes on the characters of the mature ciders, after bottling, show that in each instance the typical " Kingston Black " aroma and flavour were definitely marked. Thus there is a strong presumption that the " Kingston Black " character in those respects has its source in the fruit itself and is not due to any specific yeast growing in association with the fruit of this particular variety.

A further point of interest which emerges from the results is that they provide no evidence to show that during the course of the milling and pressing of the fruit and the subsequent transfer of the juice to the fermentation casks, there has been any noticeable infection of that juice with the yeasts from the other juices in the series milled and pressed on the same day. (The Corse Lawn and Pendock juices were expressed on November 18th, 1908, and the remaining four on November 21st, 1908). Had there been material infection of that kind, the two former juices should have shown several yeasts in common and the same applies to the other group; actually there has been remarkably little duplication between one centre and another.

A similar point can be made with regard to the possible infection of the juices by the " factory " microflora, as sometimes it has been termed. It is almost inevitable under ordinary working conditions that cidermaking premises and plant are contaminated to some degree with fermentation micro-organisms introduced from time to time in the normal course of the work. It would be expected—particularly during milling and pressing operations and the transfer of the freshly-expressed juices to the fermentation vessels—that exposure to such infection would be very considerable. Yet the results in the present cases under consideration give no indication of any general infection with types in common.

While these conclusions apply to the early fermentation stage in the histories of the ciders, the complete picture of their microbiology cannot be discussed until the details of their later life are published in a subsequent paper.

SUMMARY.

An investigation on the yeast flora of Kingston Black ciders at various stages of fermentation and maturity is described. Separate ciders were made at Long Ashton in 1908 from fruit of this variety grown at six different centres, all being given corresponding treatment.

The present paper records the results of the initial examination of the

flora of each cider at the incipient stage of fermentation, from five to eight distinct types of yeast being isolated in the respective cases.

A comparison of the yeast types obtained from the whole group of the ciders shows that no yeast common to all the juices has been found. There has been remarkably little duplication of any of the yeasts in the ciders of the various centres, each centre in effect possessing its own distinctive flora.

Thus, although four of the juices were expressed on the same day and the same processing plant used, there appears to have been no material infection of any of those juices with yeasts from either of the other three. Similarly there is no evidence of the existence of a "factory" flora dominating the ciders.

The results as a whole give no support to the view that there is any particular yeast or group of yeasts associated with the Kingston Black apple and responsible for the distinctive character of the ciders of that variety.

ACKNOWLEDGMENTS.

While the investigation described in this paper was initiated at Long Ashton, where the ciders concerned were made and kept under observation during its course and the yeasts at the respective stages isolated, the greater part of the detailed work on the characters of the individual yeast cultures was carried out in the Botany Department of the University of Bristol by arrangement with the late Professor J. H. Priestley, at that time Head of the Department. The services of Miss E. B. Pearce (Mrs. Lessimore), then his Senior Lecturer, and assistant staff were made available for the necessary tests and records. As the work progressed, the data thus obtained were examined conjointly by her and the author. As required, supplementary confirmatory observations were repeated subsequently at Long Ashton.

Without such help it would have been impossible for the very considerable cultural work associated with the examination of the characters of so large a number of yeasts to have been undertaken and carried through to completion, owing to the very limited resources in staff and equipment at Long Ashton at that time. Grateful acknowledgment of the collaboration of the Botany Department is made and in particular the author desires to express his very warm thanks to Mrs. Lessimore for her very close interest and considerable personal effort throughout the prolonged course of the work.

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STUDIES ON CIDER SICKNESS.

I. THE MOLYBDENUM CONTENT OF CIDERS IN RELATION TO THE INCIDENCE OF CIDER SICKNESS.

By B. T. P. BARKER, E. J. HEWITT and D. J. D. NICHOLAS.

INTRODUCTION

In a recent paper on this disorder of cider (1) it was stated that free growth and active motility of the causal organisms appeared to be stimulated by the presence of molybdenum (Mo) as a micronutrient in certain culture solutions. Since there was no available information on the presence or amount of this element in apple juices or ciders, it was not possible at that stage to consider if the degree of susceptibility of an individual cider to the disorder was related in any way to its molybdenum content. Hence the work described in the present paper was undertaken to determine if such correlation existed.

For the purpose of the investigation twenty-three ciders made at the Research Station during the 1948 cider-making season were selected for detailed examination. These ciders covered a wide range of types, including representatives of each of the three main classes of cider apples—sharp, sweet and bittersweet—with acid and tannin contents thus differing substantially in individual cases according to the class of the variety of apple concerned. Their sugar contents also were varied, some of the ciders being sweet, others medium-sweet and a few practically 'dry.' The rates of fermentation of the individual juices had differed considerably, denoting differences in the amounts of assimilable nitrogenous compounds originally present.

With such a range the selected ciders constitute a group in which widely varied degrees of natural resistance to sickness are represented. The most vulnerable ciders in actual practice are those which combine a high sugar content with low acidity and low nitrogen, while those with these features in converse are the most resistant. Owing to the conjoint influence of these three factors—each differing quantitatively in individual ciders—on the degree of susceptibility to sickness, it is unlikely that the molybdenum content of a cider would be a direct measure of that degree. Two alternative views as to the significance of that content are possible. The first is that, if molybdenum is an essential micronutrient, there must be an absolute minimum quantity present and, so long as that minimum is there, the chance of a sickness outbreak is determined entirely by other factors, such as those just mentioned. The other alternative is that the essential minimum is a variable amount dependent upon the degree of resistance to an outbreak conferred by other factors, the greater the degree the higher the molybdenum minimum required: in such a case the molybdenum content becomes one of the factors governing the degree of susceptibility.

The molybdenum content of each cider was determined both by the chemical method (2) and the bioassay method, using *Aspergillus niger* as the assay organism. The estimations were made at the time the 'sickness' tests recorded below were started.

SICKNESS TESTS.

Procedure.

Six "sickness" tests in duplicate were made with each cider at a temperature of 25°C. They were carried out in sterile test-tubes, 10 c.c. of the fluid under trial being used in each instance.

The treatments in the respective tests were as follows :

1. Uninoculated.
2. " : as 1, but cider neutralised by addition of excess CaCO_3 .
3. Inoculated : cider unsterilised.
4. " : after previous sterilisation of the cider.
5. " : cider unsterilised and neutralised by addition of excess CaCO_3 .
6. " : as 5, but cider sterilised before inoculation.

The inoculation consisted in the addition of one drop of an actively fermenting 24-hour-old culture of the organism in a glucose (5%) yeast extract solution : the drops were of uniform size throughout and were taken with a standardised sterile platinum-wire loop. Sterilisation of the ciders in Tests 4 and 6 was achieved by heating them at 100°C., for 5 minutes : they were then cooled immediately, prior to inoculation.

Results.

The results of the tests are shown in the accompanying Table I, together with the molybdenum content of each cider as estimated by the chemical and bioassay methods respectively.

In this Table the ciders are grouped into two classes, the first containing those of low acidity—below 0.5 per cent malic acid—and the second those of medium to high acidity—above 0.5 per cent malic acid. With three exceptions they were made from the individual varieties of apples named : the exceptions were blends of mixed varieties as named. Except in the cases starred with an asterisk, the sugar content (as indicated by the Specific Gravity) was sufficiently high to provide visible signs of fermentation by active evolution of gas bubbles of carbon dioxide, where sickness occurred : in the starred cases the sugar content was too low to give such visible signs and accordingly sufficient glucose was added to these before starting the tests to ensure adequate gas evolution in case of onset of sickness.

The first set of results to consider are those in Test 1, indicating the ciders which developed sickness without any treatment other than exposure to a temperature of 25°C. (It may be noted that each of these became 'sick' at ordinary room temperature after a longer interval of time). Of the five samples which showed undoubted sickness Sweet Alford gave the first sign of the outbreak and developed it most strongly. Yarlington Mill II also reacted strongly, while Yarlington Mill III gave a positive result more slowly in one tube, but not in the duplicate tube. Ashton Brown Jersey behaved as Yarlington Mill II, but started rather more slowly. Each of the afore-mentioned varieties belong to the low-acid group. The fifth sample, Kingston Black corresponded with Ashton Brown Jersey in time of outbreak, but the vigour was not quite so intense : this variety normally belongs to the high-acid group, but in this particular case the acidity was only slightly above the upper limit of the low-acid group. In three other ciders doubtful traces of fermentation appeared, but did not extend further : some bacteria resembling the sickness organism in appearance were present, but since yeast cells in limited number also occurred they might have been the responsible cause.

TABLE I.
RESULTS OF SICKNESS TESTS.

Ciders		Treatments and Results					
Variety	Mo Content, p.p.m.	1	2	3	4	5	6
		Uninoculated		Inoculated			
	Chemical		Neutralised	—	Sterilised	Neutralised	Neutralised and Sterilised
<i>Low Acid.</i>							
Ashton Brown Jersey	0.01	F	F-	F	—	F+	F+
Belle Norman (b)	0.01	—	—	F+	—	F tr	F-
Chisel Jersey I	0.0088	—	—	†	—	F+	F*
Chisel Jersey II (a)	0.0007	—	—	F+	—	†	†
Dabinett (a)	0.0046	—	†	—	—	F*	—
Morgan Sweet (a) (c)	0.002	—	—	F*	—	—	—
Pixley Hangdown	0.0043	—	—	—	—	F+	F tr
Sweet Alford	0.006	F+	F+	F+	—	F+	—
Woodbine I (a)	0.0063	—	†	—	—	F+	F*
Woodbine II	0.0081	—	—	†	—	—	—
Yarlington Mill I	0.002	—	—	—	—	F tr	F tr
Yarlington Mill II (a)	0.0023	F+	>F tr	F+	—	F+	F*
Yarlington Mill III	0.0015	>F tr*	>F tr	>F*	—	F+	F+
<i>High Acid.</i>							
Bramley's Seedling	0.001	—	—	—	—	F+	F*
Dymock Red	0.003	†	F	—	—	F+	F tr*
Foxwhelp	0.0065	—	—	—	—	F+	F+
Gatcombe	0.001	—	†	F+	—	F+	F*
Kingston Black	0.004	>F*	>F*	F+	—	F+	F+
Stoke Red	0.004	—	—	—	—	>F-	F+
<i>Blends (low acid)</i>							
I Broadleaf/Pertheyre	0.002	—	—	—	—	>F tr	>F tr
II Pertheyre/Sweet Alford	0.005	†	† >F*	F*	—	F	F*
<i>Blends (high acid)</i>							
III Sour Natural/Bittersweet	0.015	—	—	—	—	†	—
IV Vat 15. Mixed Fruit (b)	0.025	—	—	—	—	—	—

F+ ≡ very active fermentation.
 F ≡ moderately active fermentation.
 F- ≡ slight fermentation.
 Ftr ≡ trace fermentation.
 † ≡ doubtful trace of gas evolution.
 * ≡ fermentation onset tardy.
 (a) ≡ glucose added.
 (b) ≡ tin present.
 (c) ≡ cider ropy

The results in Test 2 followed very closely those of the first set in respect of the ciders giving positive sickness fermentations. There were, however, several additional cases of trace or moderate fermentations where yeast growth obviously had been sufficient to cause fermentation.

In both of these Tests no record of sickness has been accepted unless the micro-organism population was predominantly bacterial and identical in character with the sickness organisms.

Test 3 differed from Test 1 in treatment in direct inoculation of the ciders with the sickness organism. As a result, in addition to outbreaks of sickness in the five ciders which were similarly affected in Test 1, others occurred in Dabinett, Chisel Jersey I, Pixley Hangdown, the Sweet Alford-Pertheyre Blend II and Gatcombe. With the exception of the last-mentioned, each of these belongs to the low-acid class.

The results in Test 4—in which direct infection was preceded by sterilisation of the ciders—were completely out of line with those of the other five tests. No sign of fermentation appeared in any of the twenty-three ciders. This effect of heat in rendering them immune to infection has opened up a new line of investigation now in progress which is throwing some light on nutritional aspects of the group of sickness organisms.

The remaining two Tests, 5 and 6, had in common direct inoculation and neutralisation with calcium carbonate, and differed only in the prior sterilisation of the ciders in Test 6. Test 5 gave positive outbreak results in all except Vat 15 (a mixed blend), the Sour Natural-Bittersweet Blend III and Morgan Sweet: these special cases are discussed later. The same exceptions also gave negative results in Test 6, in which Sweet Alford, Dabinett and Woodbine II in addition failed to respond to infection. It seems that sterilisation in these three latter cases had the same effect as shown by all ciders in Test 4, notwithstanding neutralisation of their acid content, whereas similar neutralisation modified to varying degrees this sterilisation effect in the remaining seventeen ciders of Test 6, each of which became sick. In some of these seventeen ciders, e.g., Ashton Brown Jersey, there was no visible difference either in time of onset or vigour of fermentation in the two tests in question.

The three varieties which failed to give positive outbreaks of sickness in Test 5 show features which may account for that failure. In the cases of Vat 15 and the Sour Natural-Bittersweet Blend III the Mo results appear abnormally high and there is some reason for regarding this as due to contamination with tin. Belle Norman is another cider suspect in regard to some tin contamination. Morgan Sweet is another exceptional case, but of a different order. This cider showed advanced signs of ropiness. An associated investigation in progress has revealed that the sickness organism loses its motility in culture media of high viscosity and suffers a severe check in its growth. Thus with the highly viscous ropy Morgan Sweet cider it is probable that the organism could not develop sufficiently to produce active sickness.

DISCUSSION.

Molybdenum Status of Ciders.

The molybdenum content of the ciders ranged between 0.015 and 0.0007 parts per million, (omitting the case of Vat 15 where the high tin content may have resulted in a fictitiously high figure), as shown by the chemical method of determination. The corresponding extremes according to the microbiological assay method was 0.02 and 0.001 p.p.m. By both methods the cider

with the highest content was the Sour Natural-Bittersweet Blend and that with the lowest content Chisel Jersey II. In general, both methods gave approximately similar results, considering the smallness of the amounts present. In a few cases the results were markedly different, e.g., Woodbine II and Sweet Alford: this may have been due to part only of the molybdenum being available for assimilation by *Aspergillus niger*.

It is noteworthy that the cider with the lowest Mo content showed no definite fermentation under any of the six treatments. A very slight evolution of gas was observed in the uninoculated, unsterilised sample neutralised by the addition of CaCO_3 : this was most probably caused by living yeast cells present in limited number in the sample, few bacteria of any kind being observed in it under microscopical examination. A still slighter trace of gas evolution was noted in the corresponding inoculated sample: here again yeast was almost certainly the cause, since no motile sickness bacteria could be found and the cells of the inoculum appeared to be moribund or dead. Thus it may be that the Mo content in this case was below the minimum limit necessary to support growth of the sickness organism: but further evidence on this point is required before any positive claim can be established. Bearing on this point, other ciders in the tested series with Mo contents almost as low—so near to the preceding case that the minute differences may be within the range of experimental error—showed active sickness under some of the treatments: the cases of Bramley's Seedling, Gatcombe and Yarlington Mill II are examples. (See Table I for details). Accordingly, if molybdenum is an *essential micronutrient*, its minimum limit must be extremely minute and detectable only with certainty under conditions of trial considerably more stringent than those associated with a general examination such as that in the present investigation.

It will also be noted from Table I that the cider with the highest Mo content (Sour Natural-Bittersweet blend)—disregarding the abnormal case of Vat 15—failed to show any signs of sickness in any of the six tests applied. This result, however, can hardly be attributed to the molybdenum present being in toxic quantity, since in another line of work on sickness in progress it has been found that the organism will grow freely in media containing 100 p.p.m. of molybdenum, i.e. approaching 10,000 times as much as in the cider in question. It is possible, in view of the abnormally high Mo content shown by both methods of estimation as compared with all the other ciders, that the presence of some other element in toxic quantity as a contaminant may be responsible both for the apparent high molybdenum figures and the failure of the organism to grow: in that event it would correspond with Vat 15 and Belle Norman, both under suspicion for tin contamination and accompanying toxic action.

Taking the results as a whole, it is evident that there is no obvious correlation between the Mo contents of the individual ciders and their behaviour under the sickness tests applied. As pointed out earlier in the paper, the differences in composition between them in respect of other factors affecting the growth of the causal organism tend naturally to obscure any molybdenum effect and make direct comparison of that single factor impossible. What can be regarded as established by this investigation is (a) that sickness can occur when the amount of molybdenum present is a very minute trace and (b) (in conjunction with other investigations in hand), that the tolerance of the organism to relatively very large concentrations of this element is undoubted.

Influence of Locality and Variety on the Molybdenum Content of Fruit.

Cidermakers of long experience have stated repeatedly that the fruit from certain localities and orchards tends to give ciders particularly susceptible to the disorder. Doubtless the varieties of apples grown in such districts play an important part in such results, but the nature and chemistry of the soil are generally regarded as prominent factors also. Thus from the latter point of view the Mo contents of the ciders in the present trials which have originated from the same source are worth review. As it happens, there are four centres—Long Ashton, Burghill (Hereford), Faversham (Kent), and North Cadbury (Somerset)—which can be considered in this connection.

The orchards at the Long Ashton Research Station furnished the fruit for four of the ciders in the present series, the varieties being Sweet Alford, Gatcombe, Yarlington Mill and Stoke Red: the molybdenum figures as estimated by the bioassay method were respectively 0.002, 0.002, 0.002 and 0.004 p.p.m. (The bioassay results are quoted in all cases, since they presumably represent most closely the amount of molybdenum present in an assimilable condition).

From the Burghill Trial Orchard three of the ciders originated, these being Yarlington Mill II, Ashton Brown Jersey and Kingston Black with Mo contents 0.002, 0.003 and 0.004 p.p.m. in that order.

The Faversham Trial Bush Plot at Macknade provided three varieties Bramley's Seedling, Chisel Jersey and Perthyre (Blend I) with molybdenum values of 0.002, 0.001 and 0.002 p.p.m. respectively.

North Cadbury was the source of three samples—Dabinett, Yarlington Mill and Chisel Jersey, containing respectively 0.004, .005 and .01 p.p.m. of molybdenum.

These records certainly lend support to the view that the molybdenum contents of the ciders from individual centres are related to soil conditions at those places. The Faversham samples are uniformly very low in that element and those from Long Ashton and Burghill only slightly higher: the North Cadbury ciders point to a general level of a higher order, with the Chisel Jersey sample relatively very high. The latter, in particular, contrasts very sharply with that of the same variety from Faversham.

From the limited data available no conclusions on the question of possible varietal differences in respect of intake of molybdenum can be drawn. What little evidence there is tends to suggest that the varietal factor, if any, is overshadowed or obscured by the 'centre' effect.

It is noteworthy that North Cadbury is located in a district in Somerset famous for the heavy, full-bodied richness of its ciders which, however, are reputed to be particularly prone to sickness.

Heat Treatment and Neutralisation Effects.

Various associated issues of considerable interest have arisen during the course of the investigation. The most striking new one is the complete failure to induce sickness by direct inoculation with the organism in any of the ciders which have been sterilised previously by heating at a temperature of 100°C. for five minutes, (Test 4). This result indicates that such heat treatment produces some effect on the liquor which renders it immune to the growth of the organism. On the other hand, corresponding heat treatment in the presence of excess calcium carbonate to ensure neutralisation of the cider acids does not destroy the ability of the liquor to support growth of the organism. The precise nature of the vital change in the cider produced in the former case is not yet clear and is still under investigation. Reaction between the tannins of the

liquor and its Mo content, leading to removal of the whole or part of the latter from solution, may be concerned to some extent : for recent results in another section of the sickness investigations have shown a considerable lowering of the Mo content of freshly expressed juices—about 50 per cent—during their sterilisation by heat. Since, however, observations on heat treatment effects under various conditions carried on during the past twelve months show variation in the results as the ciders mature and are strongly suggestive that their nitrogenous constituents are concerned, considerable complexities are evidently involved and carry this aspect of the subject outside the scope of the present paper.

The results of the combination of heat treatment and acid neutralisation by calcium carbonate (Test 6), while showing that in the majority of cases considerable growth of the organisms occurs, nevertheless provide some evidence to indicate that the heat effect is by no means entirely eliminated by the addition of the carbonate. The comparison in this case is with the corresponding ciders in Test 5, where carbonate addition but no heat treatment has been applied. The outstanding examples of extreme difference in results are the Sweet Alford, Woodbine II, Dabinett and Pixley Hangdown ciders, where the unheated samples showed early and rapid fermentation and the comparable heated samples gave no signs of fermentation, even after a prolonged period. It is significant that each of these varieties belong to the low-acid group, the two former being of the 'Sweet' class and the two latter the 'Bittersweet' class. Less marked examples of differences in several other cases can be found on reference to the Table. In the remaining ciders no visible difference in time or vigour of fermentation was observed. The Mo content in the four named cases was not sufficiently extreme, either in the high or low direction, to suggest any obvious connection with the results.

While the Mo content thus does not appear to be directly concerned as the primary factor with the behaviour of those four ciders in relation to the combined heat and carbonate treatment, the significance of that behaviour from the point of view of the sickness susceptibility problem may prove to be of outstanding importance. Of the varieties from which those four ciders were made Sweet Alford stands out almost in a class by itself as yielding ciders remarkably prone to outbreaks of the disorder. Year after year in the course of the cider-making work at Long Ashton it has given consistently far more trouble in that respect than any other individual variety. Two of the other varieties mentioned—Dabinett and Woodbine—also have proved to be very subject to it. The fourth—Pixley Hangdown—is a little-known local Herefordshire variety, under trial at Long Ashton for the first time : further trial may show it to be another of the specially susceptible kinds. The heat-carbonate treatment in these cases, unlike the others, produced a change in the liquors which rendered them completely unable to serve as nutrient media for the growth of the sickness organism. Such change implies either the production of toxic conditions or the removal or alteration of one or more essential nutrient substances. As between the four ciders in question and the remainder it may have been a matter of degree rather than kind : for several of the latter showed some restrictive effects on growth and fermentation, although not to the extent of complete suppression.

GENERAL REVIEW OF RESULTS.

In consequence of the minute amounts of molybdenum present in some ciders which have given very vigorous fermentations in the sickness tests, the

question of that element as a micronutrient being essential for the growth of the sickness organisms can only be solved by further research under conditions where molybdenum-free culture media can be compared with corresponding media containing known amounts of added molybdenum. Such conditions are likely to be very difficult to achieve, but an attempt is being made by the use of chemical and ion-exchange methods.

Whatever may be the actual function of that element in connection with the growth of the organisms, there is now available considerable evidence of its stimulative effect. At the same time it should be stated that the impression gained by close observation of the behaviour of the organisms in the course of this and other associated investigations in progress is that, in addition to molybdenum, there are one or more other factors yet undiscovered which are essential for regular and vigorous growth. For example, in one set of experiments it was planned to ensure the presence of all probable essential trace elements in the required culture medium by the addition of a compound mixture of these, as normally used in bioassay work with *Aspergillus niger*: in the result the organisms absolutely failed to grow in spite of the presence of the other necessary nutrient substances. This pointed to a toxic effect of one or more of the trace elements in the quantities used.

Hence it is possible that sickness outbreaks in some ciders of the susceptible types might be inhibited by a corresponding excess of mineral nutrient. As previously stated, an analagous case actually occurred in the series of ciders described in this paper, where the Vat 15 samples failed to show a positive response under any of the six treatments owing possibly to excessive tin contamination. Conversely, growth failure might be due to a deficiency of a micronutrient other than molybdenum. Further work in this direction will be undertaken as soon as culture fluids of the necessary degree of purity can be prepared; when that is possible the rôle of micronutrients in relation to the disorder should be determinable.

SUMMARY.

Twenty-three ciders, made in 1948 and representing a wide range of types, have been examined to ascertain if a direct relationship between their molybdenum content and their incidence to the cider sickness disorder exists.

The molybdenum content has been determined by direct chemical analysis and the *Aspergillus niger* bioassay method. The results given by the two methods generally have been very similar. The cider with the highest content—except for one doubtful case—contained .015 and .02 p.p.m. Mo, as shown by the respective methods, and that with the lowest content .0007 and .001 respectively.

Sickness tests under six different forms of treatment have been made and have shown considerable differences in the nature of the responses of the individual ciders. In the control group, with the ciders untreated in any way, five of the samples developed unmistakeable sickness outbreaks and the remainder with one exception kept sound. At the other extreme, treatment by direct infection with an active culture of the causal organism, combined with neutralisation of the liquor by addition of excess calcium carbonate, resulted in positive outbreaks of varying degrees of vigour in all cases with two exceptions, which were due to abnormal factors.

The effect of sterilisation of the ciders by heat treatment prior to direct infection was outstanding: in no instance was sickness obtained. The other three forms of treatment gave results intermediate between the two extremes.

No apparent correlation between the Mo contents of the ciders and their behaviour in the sickness tests has been noted. Any correlation, if it exists, may have been obscured by other factors in the ciders definitely known to influence the degree of their susceptibility.

There is some evidence shown by the Mo contents of the ciders of individual varieties of apples from the various centres where the fruit was grown that the quantity of molybdenum in the juice is related to the soil conditions at those places.

One result seemingly out of line with all previous experience of the disorder was the failure of the Sweet Alford, Dabinett, Woodbine and Pixley Hangdown ciders to develop sickness after heat sterilisation and neutralisation prior to direct infection. The first three-named are among the most susceptible in actual practical cider-making and maintained that reputation in the other tests: the treatment mentioned, judging from the results with all the other ciders, should have made them still more susceptible. Further investigation in this direction is clearly necessary.

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THE CLARIFICATION OF APPLE JUICE. II. THE ESTIMATION OF PECTIC ENZYME ACTIVITY.

By MARGARET E. KIESER, A. POLLARD and AUDREY M. STONE.

INTRODUCTION.

The relative merits of clear and cloudy fruit juices have been under discussion for many years, and until the industries are completely stabilised the controversy may be expected to continue. Where the industry is well established a consumer preference has already been formed for a given type of product and in many European countries the clear type of juice is most commonly encountered. In Switzerland and in Germany apple juice has been produced mainly in a brilliant condition and this method of presentation has largely been followed in this country; in North America both clear and cloudy products are produced on a considerable scale. The clear juices are, for the greater part, obtained by the use of filtration enzymes derived from mould cultures, but in France clear juices have been produced by other methods involving spontaneous clearing by the fruit enzymes themselves and this procedure has been discussed elsewhere (1); attention there would, however, now seem to be turning towards the more generally used type of enzyme clarification.

The growth of the apple juice industry has stimulated the production of clarification enzymes in each country and their preparation and use has called for methods for evaluating their activity and applicability. Studies of the factors involved in the clarification of fruit juices were made in Germany in the early days of the industry and Mehltitz and his collaborators published an extensive series of papers. Since that time the work has been developed further, mainly

in Switzerland and in the United States. The present position has been summarised by Reid in the section on Pectic Enzymes in the new Technical Communication No. 21, Recent Advances in Fruit Juice Production (2), where the uses of pectin degrading enzymes and the underlying theoretical problems are discussed in detail with the appropriate bibliography.

The section which follows gives a description of a viscosimetric method for the comparison of the activities of pectic enzyme preparations, together with a discussion of its applicability. As the theoretical background has been fully dealt with in the article mentioned above, it will only be briefly outlined in the present account.

The measurement of enzyme activity

The evaluation of the activity of clarification enzyme preparations is complicated by the fact that they commonly contain variable proportions of both pectin esterase (PE) and polygalacturonase (PG) while the juice itself may contain pectin esterase not necessarily identical with the first. The effect to be measured is essentially that of the PG on the pectin in solution. To eliminate the effect of the accompanying pectin esterase, which facilitates the action of the PG, Weber and Deuel (3) suggested the use of pectic acid as a substrate rather than pectin itself. As a nearer approximation to the conditions found in actual practice they estimated the enzyme activity in a solution of pectin in a de-pectinised and heated juice or diluted concentrate.

Of the methods available for estimating the amount of breakdown actually produced in the pectin molecule, one of the simplest is the estimation of viscosity change. As shown by Mehltz and Scheuer (4) this change in viscosity can be used to obtain a general estimate of the efficiency of an enzyme preparation, but, as described by Reid (2) and as will be further shown by the results to be described, the method has limitations. A convenient estimate of viscosity change is given by the "Abbau-zahl" (A) of Weber and Deuel, i.e. the percentage fall in pectin viscosity (measured in seconds) produced by the enzyme in a given time.

$$A = \frac{t_a - t}{t_a - t_o} \cdot 100$$

where t_a = viscosity at the start.

t_o = viscosity of the pectin-free solution.

t = viscosity after given enzyme action.

The relationships between pectin concentration and viscosity, or between enzyme concentration and viscosity or pectin breakdown, are not linear, but the measurement of viscosity changes produced by two given enzyme preparations can be used to give a relative comparison between them. By standardising the conditions and by keeping either the substrate or one enzyme constant the comparison may more readily be obtained, for the concentration of enzyme needed to produce a given fall in viscosity can then be estimated. It was observed empirically by Hooper and Ayres (5) that the relation between viscosity fall and the logarithm of the enzyme concentration is linear over a considerable range, and this enables the comparison of different enzyme preparations to be made more readily. A method of test based on this observation has recently been described by Reid (2; 6) where a standard enzyme preparation has been used for comparison. The essential difference between this method and our own is that in our own tests we have endeavoured to standardise both the pectin substrate and the experimental conditions to

avoid the need for maintaining a standard enzyme, the pectin preparations being essentially the more stable. A simple graphical method has been used for computing the results.

EXPERIMENTAL.

Method of Test.

All solutions were made up in citrate buffer pH 3.5 (0.25 M) and viscosity measurements (Ostwald viscometer) were taken after a constant time of 4 hours reaction at 25°C. The pectin substrate was a dry preparation of apple pectin made up under constant conditions in citrate buffer with sodium benzoate (0.125%). This solution (0.5% pectin) was diluted to 2/3 concentration in the final reaction mixture.

All enzyme solutions were prepared by extracting the preparations in buffer solution for 2 hours at 30°C with frequent agitation; they were then

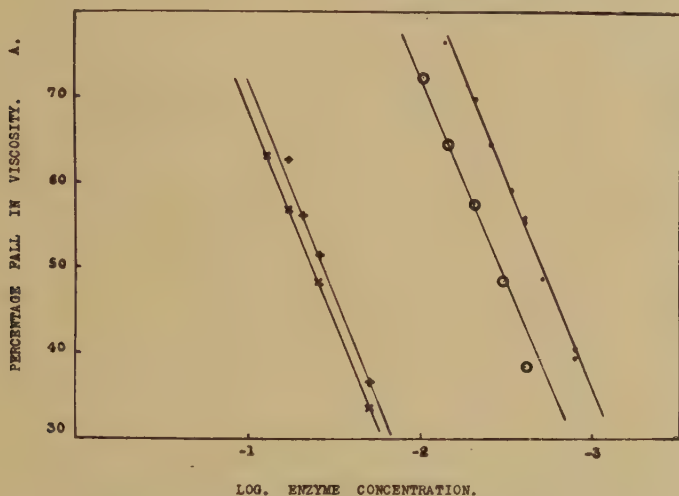


Fig. 1. The effect of enzyme concentration on the rate of pectin breakdown as measured by percentage fall in viscosity (A). Comparison of four enzyme preparations.

filtered through coarse paper for use. As a standard for comparison use was made of an enzyme preparation which had been kept at room temperature for a number of years and which still showed high activity. It was assumed that the preparation would show little further change over the course of the tests; moreover this material was completely dispersible in water and the solution of the enzyme presented no difficulty.

Several enzyme preparations were tested and the fall in viscosity of standard pectin solutions was noted for different concentrations of enzyme. The percentage fall in viscosity (A) plotted against the logarithm of the enzyme concentration gave a series of straight lines as shown in the Figure, the range of A being from about 30 to 70%. In this diagram the actual concentration of enzyme decreases from left to right. These lines were used as a guide in estimating the enzyme concentration required to give a standard change in viscosity equivalent to $A = 50\%$. This value was chosen as a midway point showing the least variation due to any change in the slope of the lines due to experimental errors. The degree of reproducibility of the method is shown by the following independent estimates obtained for two enzyme preparations in tests carried

out at intervals of several months :—Concentration required for A = 50% :—Standard Preparation 1, 0.00193% (1948), 0.00198 (1949); Preparation 2, 0.0036% (1948), 0.0038% (1949).

It will be evident that the behaviour of an enzyme in a pure pectin (or pectic acid) substrate will not necessarily be paralleled in a juice where complicating factors may be present. In the present tests estimates of activity as described above have been followed by tests of the same preparations in apple juices and pectin-juice mixtures. In the juice tests the activities were compared by estimating the minimum concentration of enzyme needed to clear the juice and this usually followed closely the change in viscosity, except where fining agents had been incorporated in the preparations. The relative activities of a number of preparations as estimated by the two methods are shown in Table I.

TABLE I.
COMPARATIVE ACTIVITIES OF ENZYME PREPARATIONS.

Preparation	By Pectin Test	By Juice Test
No. 1 (standard)	100	100
No. 2	54	69
No. 3	5	7
No. 4	4.5	12
No. 5	ca. 117	nil

Preparations 2 and 3 show relative activities in juice of the same order as those found in pure pectin solutions, but Preparation 4, containing additional fining agents shows a higher clarifying power in juice. Preparation 5 is of special interest in showing a very high activity in pectin but a negligible activity in apple juice. This preparation will be discussed in greater detail below.

Preparation 5. This material, a highly purified enzyme preparation from *Penicillium brevicompactum*, was provided by the University of Belfast and it was of particular interest in showing an almost complete inactivation in apple juice or juice mixtures. Preparations 1 and 2 had almost the same relative activities in pectin solution and in the juices tested and the amount of enzyme needed to clear a juice overnight was for each enzyme about five times the concentration needed to give a 50% reduction in the viscosity of the test pectin solution. Preparation 5, however, which was more active than either of these in pectin solution, produced no clarification of a juice in a twenty-fold concentration. It was first thought that Preparation 5 might be lacking in pectin esterase, though this would only account for its inactivity in juice if the juice pectin were more highly methylated than the test pectin and the juice itself lacked pectase. The addition of tomato pectase to Preparation 5 did in fact give no increase in clarifying activity.

That the behaviour of the material in juices was rather due to an inactivation by juice constituents was shown by tests on solutions of the test pectin in a diluted concentrate prepared from depectinised apple juice. In this solution the enzyme had only about one hundredth of the activity shown against the same pectin in buffer solution. The inhibitory effect of tannin was described by Mehltz and Maass (7) who found a gradual decrease in the amount of pectin

degradation produced by enzyme systems as the amount of tannin in a juice increased. In the present tests later batches of Preparations 5 and 2 were tested in pectin solutions to which tannic acid had been added and also in juice which had been treated with gelatine to reduce its tannin content.

TABLE II.
EFFECT OF TANNIC ACID ON PECTIN DEGRADATION.

Tannic acid added to pectin solution	Relative activities of enzyme preparations	
	No. 2	No. 5
0.0%	100	100
0.1%	86	63
0.3%	65	26

As shown in Table II the addition of tannic acid to the pectin solution gave a considerable reduction in the activity of No. 5, the higher rate of addition reducing the activity to 26% of its original value; No. 2 showed the same effect but to a less marked extent.

In the tests where gelatine was added to precipitate juice tannin the addition (0.03%) was made when or before the enzyme was added: when added first, the gelatine-tannin precipitate was removed by the centrifuge. The course of the enzyme action as measured by viscosity change is shown in Table III.

TABLE III.
EFFECT OF TANNIN REMOVAL ON JUICE CLARIFICATION AS MEASURED
BY CHANGE IN VISCOSITY (TIME IN SECONDS).

Juice	Control (no enzyme)	Prepn. 2		Prepn. 5	
		0.02%	0.04%	0.02%	0.04%
(a) Gelatine before enzyme	296	89	73	295	292
(b) Gelatine with enzyme	298	85	72	304	296
(c) No gelatine	296	86	73	309	297

(Water: 40.5)

It will be seen that the gelatine additions did not influence the course of clarification, Preparation 2 again reduced the juice viscosity and cleared the juice at both rates of enzyme addition, while Preparation 5 still proved inactive and the viscosity remained unchanged.

In a second test a larger amount of gelatine was added to a juice, sufficient to reduce the juice tannin (as estimated by permanganate titration) from 0.25% to 0.12%. The effects of adding the same two enzyme preparations to this juice are shown in Table IV, where the changes in viscosity are listed.

TABLE IV.
ACTIVITY OF ENZYMES IN JUICE AFTER GELATINE TREATMENT.

Enzyme addition	Viscosity (time in sec.) after 20 hours.	Percentage fall in viscosity
nil	131	—
Prepn. 2 (0.02%)	64	94
„ „ (0.04%)	61	(taken as) 100
Prepn. 5 (0.02%)	121	14
„ „ (0.04%)	110	30

At both concentrations Preparation 2 gave complete clarification of the juice and the viscosity was reduced; the other preparation gave some reduction in viscosity and a partial clarification, but the extent of the change was still relatively small.

Preparation 5 thus shows a higher sensitivity to the presence of tannin material than the other enzymes tested and this offers, at least in part, an explanation of its low activity in apple juice or juice mixtures. It would be expected that a highly purified enzyme preparation of this type would be more readily inactivated by the formation of a tannin-protein complex than would a less purified commercial product.

DISCUSSION.

A simple viscosity test of the type described above can thus give a useful indication of the activity of an enzyme preparation when its clarifying power is directly due to its enzyme content and in the absence of interfering factors. It is of value in following the activity of different batches of a given preparation of known general properties. When an enzyme is combined with other clarifying agents then such a test will give too low an estimate of the effect actually produced in a juice. Conversely, a viscosity test in pure pectin solution will give a false estimate of clarifying power if the enzyme preparation is readily inactivated by juice constituents. Estimates of the activity of commercial preparations would, in any case, be most fairly related to their cost to the user and be expressed on a basis of unit cost rather than unit weight of material.

Tests of enzyme activity carried out in juices themselves will still show some variability due to differences in juice composition. The effect of tannin has already been mentioned, but in addition there will also be differences due to variations in pH, pectin content and the activity of the fruit pectase. These factors will vary according to the variety of fruit and its state of maturity and the investigation of their effects is in progress. The experiments described in a previous Report (1) have shown that apple varieties vary greatly in their content of pectase and this may in turn affect the course of juice clarification. Further work on the occurrence and properties of the fruit enzymes will be reported later.

SUMMARY.

A description is given of a method for the viscosimetric assay of pectic enzyme preparations in which the substrate and conditions of test are standardised.

The applicability and limitations of the method are discussed.

The activities of enzyme preparations as found by this method are compared with the clarifying effects actually produced in apple juice and some of the factors involved are discussed.

ACKNOWLEDGMENT.

We wish to express out thanks to Miss M. E. Moseley for assistance in the experimental work.

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THE EFFECT OF MANURIAL TREATMENT ON THE COMPOSITION OF BLACKCURRANTS AND THEIR PRODUCTS

By MARGARET E. KIESER, A. POLLARD and AUDREY M. STONE.

Some of the effects of the nitrogenous manuring of blackcurrants on the composition of the fruit have been described in a previous Report (1). In the two varieties studied, viz. Mendip Cross and Cotswold Cross, a relation was found between the level of nitrogen taken up by the bush and the level of ascorbic acid and sugar in the fruit. The effect was the more marked in Cotswold Cross, and at the higher levels of nitrogen uptake there was a decrease in the amounts of ascorbic acid and sugar. But, in terms of total ascorbic acid production, the increase in yield following nitrogenous manuring more than counterbalanced the lower level in the fruit.

Further tests have been carried out on this plot during the past two years. In 1948 the replicate plots were again sampled individually; in 1949 the samples were used for the preparation of juice and syrup, to determine whether any difference in fruit composition was maintained during normal processing.

EXPERIMENTAL.

1948 Trials.

Samples were drawn from each replicate plot as in previous years and representative samples from each batch were analysed for ascorbic acid, sugar and free acid. The values found for these constituents showed no definite relationship with the nitrogen status of the bushes and some differences in fruit composition appeared to be due to site effects. The differences in nitrogen status of the bushes on the different manurial plots were also less

marked than in the previous season. The results will not be given in detail, but some reference to the general levels of ascorbic acid will be made later.

1949 Trials.

In these tests each individual bush of the variety was sampled by taking five complete strigs in firm ripe condition. All fruit from a given manurial treatment was combined to give a bulk sample of about 5 lb. for each treatment. Each bulk was milled and samples were withdrawn immediately for analysis of ascorbic acid and pectin; the remainder of the milled fruit was treated with Pectozyme (0.3%) and the juice pressed out after 24 hours. Syrups were prepared from these juices by adding to each the same proportion of sugar in amount sufficient to give a final specific gravity of about 1.26. The exact specific gravities of the final syrups showed slight variations according to the specific gravities of the original juices. The syrups were bottled and pasteurized and their ascorbic acid contents were determined after two weeks.

The analyses of the fruit samples, juices and syrups are shown in Table I and for the purpose of comparison they are arranged in order of increasing nitrogen status of the plots. The values for pectin, sugar and free acid show no marked differences to be attributed to manurial treatment. In Cotswold Cross the tannin content and the specific gravity of the juice appear slightly higher in the fruit from the lower nitrogen plots, but in Mendip Cross there is no consistent difference.

As in some of the previous tests, the ascorbic acid values for Cotswold Cross show a definite fall with increasing uptake of nitrogen by the bush. The effect is more marked in the juice than in the fruit itself, for the losses of ascorbic acid during juice extraction appear greater where the level is initially low. The figures for the syrups fall in practically the same order as those for the fruit and the difference between the highest and lowest figure is again greater in proportion than in the fruit. In Mendip Cross the ascorbic acid figures show a general trend for a low level to be associated with a higher uptake of nitrogen. A comparison of the ascorbic acid figures for fruit and juice show a much greater loss on juice extraction in Cotswold Cross than in Mendip Cross, but it is not clear from the present tests whether this is an effect of variety or of differences in the condition of the fruit.

DISCUSSION.

It is apparent from the 1949 tests and from those of previous years that manurial treatment can affect the composition of the blackcurrant, but in only one of the two varieties tested has the effect been fairly consistent, nitrogenous manuring giving a lower ascorbic acid content. This effect has been offset by an increase in yield and by an improvement in the nutrition of the bushes. The individual fruits from bushes receiving adequate supplies of nitrogen have also been rather larger than those of low nitrogen status and this would offer an explanation for their lower ascorbic acid content, for in smaller fruits the proportion of skin to pulp is greater and ascorbic acid has always been found in higher concentration in the skin. An increase in the size of the berries should lead to an increase in the yield of juice on pressing and in the 1949 tests the batches of Cotswold Cross from the two highest nitrogen plots gave a slightly higher yield than the other batches.

The differences in ascorbic acid content of the fruit which have been noted in the past four years are summarised in Table II, all values being given as mg. per 100 g. fruit.

TABLE I.
RELATIONSHIP BETWEEN NITROGEN STATUS OF BUSHES AND THE COMPOSITION OF FRUIT, JUICE AND SYRUP.
COTTONWOLD CROSS

Plot No.	N. status of bushes (leaf N%)	Ascorbic acid in Fruit (mg/100 g)	Ascorbic acid in Juice (mg/100 g)	Ascorbic acid in Syrup (mg/100 g)	Pectin in Fruit (as % calcium pectate)	Acid in Juice (as % citric)	Total Sugar in Juice (as % invert)	Tannin in Juice %	Specific gravity of Juice
6	2.51	199	174	90.3	1.56	3.10	4.23	0.42	1.059
1	2.56	194	173	92.0	1.25	3.14	4.24	0.42	1.058
3	2.70	189	158	82.3	1.33	3.10	4.23	0.39	1.059
2	2.86	187	158	83.3	1.69	3.10	4.18	0.40	1.058
4	2.87	186	151	80.2	1.47	2.95	3.91	0.37	1.055
5	2.90	179	146	76.3	1.43	2.98	4.23	0.38	1.055

MENDIP CROSS

Plot No.	N. status of bushes (leaf N%)	Ascorbic acid in Fruit (mg/100 g)	Ascorbic acid in Juice (mg/100 g)	Ascorbic acid in Syrup (mg/100 g)	Pectin in Fruit (as % calcium pectate)	Acid in Juice (as % citric)	Total Sugar in Juice (as % invert)	Tannin in Juice %	Specific gravity of Juice
6	2.40	212	197	98.9	1.28	3.38	3.87	0.42	1.056
1	2.47	221	210	97.3	1.34	3.77	4.00	0.43	1.058
4	2.54	212	188	89.3	1.24	3.41	3.96	0.40	1.055
3	2.56	208	200	96.4	1.21	3.51	3.84	0.40	1.057
2	2.72	199	189	94.0	1.25	3.80	3.93	0.42	1.056
5	2.82	209	178	86.4	1.31	3.51	3.59	0.40	1.053

TABLE II.
VARIATIONS IN ASCORBIC ACID CONTENT IN THE FRUIT OF TWO BLACKCURRANT
VARIETIES.

Year	Cotswold Cross		Mendip Cross	
	Average	Maximum difference due to manurial treatments	Average	Maximum difference due to manurial treatments
1946	163.5	10.5	176.6	15.0
1947	141.1	27.2	170.1	13.0
1948	182.6	27.3	216.8	21.6
1949	188.9	19.3	210.2	21.7

Maximum difference between yearly averages :—

Cotswold Cross 47.8

Mendip Cross 46.7

Maximum difference between varieties in any year :—

34.2

It is apparent that the differences in ascorbic acid which can be ascribed to manurial effects in any year have been less than those due to yearly variation within a variety or to differences between the varieties. Of the three factors seasonal variation, variety and nutrition, only the last two come within the influence of the grower. Applications of nitrogen may produce a lowering of ascorbic acid in the fruit, but yield is increased and the fruit is better in general quality. There would thus appear to be no overall disadvantage in giving adequate supplies of nitrogen : high ascorbic acid production may be best ensured by the choice of varieties with intrinsically high ascorbic acid levels.

SUMMARY

Further tests have been carried out on the effect of the nitrogenous manuring of blackcurrants on the composition of the fruit.

An increase in the uptake of nitrogen by the bush has been found to be associated with a lowering of the ascorbic acid content of the fruit, but the two varieties studied have not shown the effect consistently. Nitrogenous manuring has, however, given fruit of better size and general quality.

It is considered that ascorbic acid production may best be ensured by the choice of varieties with naturally high ascorbic acid content and the maintenance of a high cultural level.

ACKNOWLEDGMENTS.

We are indebted to Dr. C. Bould and Mr. E. Catlow for the nitrogen figures quoted in Table I and we wish to express our thanks to Miss M. R. Moseley for assistance in the experimental work.

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THE INACTIVATION TEMPERATURES OF THE OXIDASE ENZYMES IN PLUMS AND APPLES

By ALICE CRANG and LORNA KENDALL.

Previous work on the enzyme system concerned with the browning of processed pears (1) showed that processing temperatures used in bottling, which were considered sufficient to prevent growth of micro-organisms, did not necessarily inactivate the oxidase, so that the fruit turned brown on exposure to the air. As the same trouble had arisen with certain varieties of plums, it was decided to investigate the temperature of inactivation of the enzyme systems in plums and in apples.

EXPERIMENTAL.

Twelve varieties of fresh plums and nine of apples were tested. Most of the plums were examined at the under-ripe, firm-ripe and fully ripe stages, and the apples were slightly under-ripe. Twelve further varieties of plums were examined for browning on exposure to the air after processing at 165° F.

Chemical Methods.

(a) The fruit was cooled in a refrigerator and macerated on ice. The pulp was quickly transferred into small test tubes to the depth of half an inch, corks loosely inserted and the tubes placed in water at 110° F. The temperature was raised at the rate of 10° in three minutes, tubes being taken out at regular intervals and cooled in iced water. The effect of holding the pulp for varying lengths of time at temperatures just below that needed for instantaneous destruction of the enzyme was also tested.

The presence of oxidase was demonstrated by a blue-purple colouration developing on the addition of a 1% alcoholic solution of benzidine to the pulp. A further series of tests was carried out adding hydrogen peroxide as well as benzidine, but the blue-purple colouration did not develop at a higher temperature although the reaction took place more rapidly. This is in contrast to the results obtained with pears (1) in which the "oxidase," tested with 1% benzidine or guaiacol, was inactivated at a lower temperature than the "peroxidase," demonstrated on the addition of hydrogen peroxide.

(b) The degree of browning of the fruit pulp, heated to different temperatures, was noted after exposure to the air on a white tile.

(c) The temperatures attained during processing were tested by means of a thermo-couple, with the probe inserted in the fruit in the centre of the container.

Processing Methods.

The plums were placed into 1 or 2 lb. screw-band type of jars, filled with cold sugar syrup and immersed in cold water.

Two temperatures of processing the jars were compared:—

(i) Raising the water bath to 165° F. in 1½ hours and holding it at that temperature for 10 minutes before removing and cooling the jars in the air.

(ii) Raising the water bath to 180° F. in 1½ hours and holding it for 15 minutes before cooling as above.

In other cases the fruit was placed in A2½ sized fruit lacquered cans,

filled with boiling syrup to within $\frac{3}{8}$ " of the top, sealed and processed in boiling water. Time of processing was in most cases 3 to 5 mins., to reboil the water, and holding for 15-16 minutes before cooling in cold running water.

RESULTS.

Plums.

The degree of oxidase activity varied considerably with the variety. The temperature of inactivation of the enzyme increased in some varieties as the fruit ripened from the under-ripe to the firm-ripe stage, being about 10° higher in the latter. This is similar to the results found with pears (1).

In each case there was excellent correlation between browning of the fruit pulp on exposure to the air and oxidase activity denoted by blue colouration with benzidene.

Table I shows the degree of enzyme activity of ripe plum pulp brought up to various temperatures and cooled immediately afterwards.

TABLE I.
THE EFFECT OF TEMPERATURE ON THE OXIDASE ACTIVITY OF PLUM PULP.

Variety	Oxidase Activity							
	130° F.	140° F.	150° F.	160° F.	170° F.	175° F.	180° F.	190° F.
Apricot Gage	++	++	+	+	—			
Belle de Louvain	++	++	+	+	trace	—		
Blaisdon Red	++	++	+	trace	—			
Frome Cross	++	++	+	+	—			
Golden Gage	++	++	+	+	trace	—		
Pershire, Yellow Egg	++	+	+	—				
Pershire, Purple	++	+	trace	—				
Pond's Seedling	+++	++	+	+	trace	—		
Severn Cross	+++	+++	+++	++	+	trace	—	
Shropshire Prune Damson	+++	+++	++	+	—			
Thames Cross	+++	+++	+++	+++	++	++	++	—
Victoria	++	++	+	+	trace	—		

The enzyme system in Golden Gage plums was not inactivated after 30 minutes at 160° F. There was a trace of enzyme activity in the Frome Cross pulp after holding for 5 minutes at 160° F., but none after 10 minutes or longer.

Table II gives a comparison of the browning occurring in plums, processed at different temperatures, after opening the jars and leaving the fruit in a dish overnight.

The maximum temperature attained by the fruit in the centre of each container averaged 159.3° and 175° in the jars processed at 165° and 180° F. respectively.

TABLE II.

THE EFFECT OF PROCESSING TEMPERATURE ON THE BROWNING OF BOTTLED PLUMS.

Variety	Maximum Temperature of Water Bath		
	165° F.	180° F.	212° F.
Apricot Gage	+	—	—
Belle de Louvain	+	—	—
Blaisdon Red	±	—	—
Coe's Golden Drop	+++		
Count d'Althann's Gage ..	+		
Czar	—		
Frome Cross	±	—	—
Giant Prune	—		
Golden Gage	++		
Jefferson's Gage	+++		
Kentish Bush	++		
Laxton's Bountiful	+		
Laxton's Cropper	+		
Laxton's Gage	+++		
Marjorie's Seedling	+		
Monarch	+		
Pershire, Yellow Egg	±	—	—
Pershire, Purple	—	—	—
Pond's Seedling	+		
Severn Cross	+++	++	—
Shropshire Prune Damson ..	+	—	—
Thames Cross	+++	+++	—
Victoria	++	—	—
Warwickshire Drooper	+		

Apples.

The following varieties of apples were tested during the first week of November, when at the slightly under-ripe stage. The enzyme reaction was tested with benzidene and hydrogen peroxide on pulp heated to different temperatures.

It will be seen that the temperature at which the oxidase enzyme in the apple is inactivated is between 150° and 160° F. in the varieties tested.

It has been noted that apple pulp stored in hermetically sealed glass containers normally retained its colour very well, but the same pulp stored

TABLE III.
THE EFFECT OF TEMPERATURE ON THE OXIDASE ACTIVITY OF APPLE PULP.

Variety	Oxidase Activity			
	140° F.	150° F.	160° F.	170° F.
Allington Pippin	++	+	trace	—
Blenheim Orange	++	+	trace	—
Bramley's Seedling ..	++	+	—	—
Cox's Orange Pippin ..	++	+	trace	—
Crawley Beauty	+++	++	trace	—
Edward VII	trace	trace	—	—
Laxton's Superb	trace	trace	—	—
Newton Wonder	+	—	—	—
Winston	++	trace	—	—

in jars covered with a plastic material sold for the purpose turned brown. Rosoff and Cruess (2) have noted apparent regeneration of the peroxidase in frozen vegetables on standing at room temperatures, although the vegetables had been blanched to destroy this enzyme before freezing. The apple pulp was therefore tested for oxidase or peroxidase activity with both guaiacol and benzidine, with negative results. As the plastic material had been found previously to be partly permeable to oxygen, it would seem that browning occurring in apple pulp stored for some time in the presence of a small amount of oxygen is due to oxidation of the tannin without any enzyme activity.

CONCLUSIONS.

The enzyme system concerned with the browning of plums was inactivated at temperatures varying between 150° and 185° F. In fruit bottling, one standard method which has been recommended for some years was processing at 165° F., but since the average maximum temperature attained by the centre fruit processed under these conditions is only 159.3° F., this is insufficient to destroy the enzyme in most varieties of plums, with consequent browning of the fruit on exposure to the air. If, however, the temperature of processing is increased to 180° F. for 15 minutes giving an average maximum of 175° F. in the centre fruit, the only varieties so far found with an enzyme capable of surviving that temperature are Severn Cross and Thames Cross, both of which are really dessert fruits. The increase in temperature to 180° F. is therefore being recommended in future for this method of bottling plums.

The browning of bottled apples sometimes occurs during storage. It seems that this only occurs when oxygen is present, due to the use of covers which are permeable. No trace of enzyme activity was found after processing bottled apples at 165° F., and there was no indication of regeneration of the enzyme on storage.

SUMMARY.

The temperature necessary to inactivate the enzyme system responsible for the browning of plums and apples has been investigated. It is found that in plums this varies from 150° to 185° F. with the different varieties

tested. The canning processes are quite sufficient to inactivate the oxidase, but an increase in processing temperature for some bottling methods is necessary, otherwise severe browning of the fruit on exposure to the air may occur. The oxidase system in apples was inactivated in all the varieties tested between 150° and 160° F., hence the present processing times recommended for bottling this fruit should be adequate.

ACKNOWLEDGMENTS.

Our thanks are due to Mr. J. Chadderton for assistance in testing the plums, and to Miss M. Sturdy for tests on the permeability of covers.

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AIR TEMPERATURES AT LONG ASHTON, 1920—1949.

By G. E. CLOTHIER

Records of air temperatures at Long Ashton Research Station have been taken since 1920, and the figures for the thirty years from that date to 1949 have been summarised.

The equipment of the meteorological station includes two "ordinary" thermometers used as dry and wet bulbs, together with a maximum thermometer and a minimum thermometer of the "sheathed" pattern placed four feet above the ground in a Stevenson screen. A "sheathed" pattern thermometer exposed over a grass surface is used to determine the number of "ground frosts" at night. Records are taken daily at 0900 hours G.M.T., at which time the self-recording thermometers are set. The situation of the meteorological plot has already been described (1).

Annual Temperature.

The mean annual temperature at Long Ashton for the period 1920 to 1949 is 50.1°F. Fig. 1 shows the variations in mean annual temperature during that time. In common with all parts of the British Isles, the variations are relatively small, and in only five years does the value differ by more than 1.0° from the normal, the highest being +2.1° in 1921, and the lowest -1.4° in the following year, 1922.

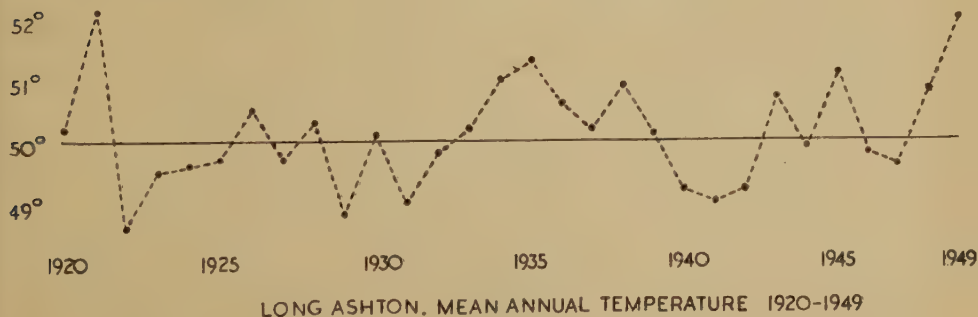


Fig. 1.

TABLE I.
AIR TEMPERATURE AT LONG ASHTON, 1920-1949.
MONTHLY AND ANNUAL MEAN TEMPERATURES (°F)

Year	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
1920	43.5	44.9	46.5	47.6	52.9	57.3	58.1	58.1	55.8	52.1	45.0	41.0	50.1
1921	47.6	41.5	46.1	48.3	53.4	60.0	66.7	60.5	58.6	56.5	43.0	44.1	52.2
1922	41.1	41.9	44.7	43.4	55.8	57.5	57.6	56.7	55.0	47.4	42.8	43.5	48.7
1923	42.1	44.9	44.7	46.9	50.1	64.3	59.5	60.9	55.3	56.3	38.5	41.0	49.6
1924	42.1	38.5	41.4	46.2	53.7	57.5	59.5	57.7	57.3	51.8	45.6	45.1	49.7
1925	43.0	42.8	41.7	46.4	53.3	60.1	62.9	61.5	53.9	52.7	40.1	38.8	49.8
1926	41.5	46.7	44.3	48.7	51.1	56.9	63.3	62.7	60.2	47.5	43.9	40.4	50.6
1927	41.8	40.3	46.5	47.4	54.1	55.9	61.5	60.9	56.1	52.1	44.7	37.2	49.9
1928	42.6	43.4	44.3	47.9	53.2	57.2	60.7	60.7	55.5	52.1	47.5	38.1	50.4
1929	35.3	32.9	44.3	44.5	52.5	55.8	61.5	59.9	60.9	50.1	45.1	43.6	48.9
1930	43.1	37.3	42.4	48.4	51.7	60.9	60.2	61.5	57.9	52.5	44.9	41.7	50.2
1931	38.9	40.5	41.0	46.8	52.8	59.7	59.4	58.8	53.5	48.5	46.9	42.3	49.1
1932	44.1	37.7	41.2	45.5	51.3	58.5	60.7	65.1	56.4	49.8	44.9	43.1	49.9
1933	37.1	40.1	46.5	48.6	55.1	60.5	64.3	64.3	59.4	51.0	42.8	34.1	50.3
1934	40.5	38.5	42.4	47.0	53.1	60.6	65.8	59.8	58.5	52.0	44.3	47.7	50.9
1935	41.7	43.7	44.5	47.8	51.5	59.6	63.7	62.3	56.9	50.5	44.5	38.6	50.4
1936	41.1	37.8	45.8	44.1	53.3	59.3	59.7	61.7	58.5	49.1	42.9	42.9	49.7
1937	42.9	43.7	39.6	49.9	54.5	57.7	60.8	63.5	57.0	51.3	41.7	38.7	50.1
1938	43.8	44.1	47.7	46.3	52.3	58.1	59.5	61.1	56.9	51.9	49.5	40.5	51.0
1939	41.5	42.9	44.0	48.0	53.2	58.1	59.3	61.9	58.5	48.1	48.9	38.5	50.2
1940	39.7	39.5	44.2	48.9	55.1	61.9	58.7	61.4	55.5	50.1	45.7	39.7	49.3
1941	34.8	40.5	42.3	44.6	48.4	59.4	62.6	59.2	58.5	51.3	45.2	43.0	49.1
1942	35.7	33.7	43.1	49.5	52.1	58.5	62.7	61.3	57.0	51.4	40.1	46.0	49.3
1943	43.4	43.3	44.7	51.8	53.7	57.9	62.3	60.6	56.0	51.9	44.3	39.4	50.8
1944	43.7	39.2	42.5	50.3	53.2	57.3	61.6	63.3	54.9	49.7	44.8	40.1	50.1
1945	34.3	45.5	46.6	51.6	54.3	57.8	61.4	61.4	58.4	53.9	45.9	43.1	51.2
1946	38.0	42.5	45.7	50.5	51.6	55.1	61.5	58.4	57.5	50.6	48.1	38.7	49.9
1947	37.2	29.1	40.3	48.0	55.5	59.8	62.1	65.4	59.0	51.2	46.5	42.1	49.7
1948	43.2	41.5	48.0	48.2	53.7	57.1	60.8	60.0	57.9	50.8	47.3	43.2	51.0
1949	42.9	42.7	42.7	50.6	52.5	60.3	64.6	63.1	61.5	54.9	44.8	43.6	52.1
Average	40.6	40.7	43.9	47.8	53.0	58.4	61.7	61.1	57.3	51.1	44.7	41.4	50.1

Figures in bold type indicate the largest and smallest monthly and annual means.

TABLE II.
AIR TEMPERATURES AT LONG ASHTON. 1920-1949.
MONTHLY MEANS, WITH EXTREMES AND YEARS IN WHICH THEY OCCURRED. (°F).

	January	February	March	April	May	June	July	August	September	October	November	December
Monthly Mean	40.6	40.7	43.9.	47.8	53.0	58.4	61.7	61.1	57.3	51.1	44.7	41.4
Highest Monthly Mean Year	47.6. '21	46.7 '26	48.0 '48	51.8 '43	55.8 '22	61.9 '40	66.7 '21	65.4 '47	61.5 '49	56.5 '21	49.5 '38	47.7 '34
Lowest Monthly Mean Year	30.7 '40	29.1 '47	39.6 '37	43.4 '22	48.4 '41	55.1 '46	57.6 '22	56.7 '22	53.5 '31	47.4 '22	38.5 '23	34.1 '33
Extreme range	16.9	17.6	8.4	8.4	7.4	6.8	9.1	8.7	8.0	9.1	11.0	13.6
Mean Daily Maximum ..	46.2	46.4	51.6	55.6	61.7	67.0	69.6	69.2	65.2	58.1	50.7	46.7
Mean Daily Minimum ..	35.1	35.2	36.6	40.0	44.3	49.8	52.6	53.1	49.4	44.1	38.7	36.1
Mean Daily Range	11.1	11.2	14.6	15.6	17.4	17.2	17.0	16.1	15.8	14.0	12.0	10.6
Highest Day Temperature	59	62	72	76	84	87	93	93	83	80	66	59
Lowest Night Temperature	8	6	13	25	25	35	41	38	33	24	21	18

Monthly Temperature.

Table I shows the mean monthly temperature in °F month by month for each of the years 1920–1949, together with the average for each month and year, while in Table II averages and extremes, and the years in which they occurred, are set out.

The average daily range of temperature is about 11° from December to February, rising to a maximum of 17.4° by May, followed by a slow decline during the summer months before falling sharply from September onwards to the winter minimum.

Though the daily range of temperature is least during the winter months, the figures show that the largest departures from the monthly means are to be found at that time of the year. The means are not evenly distributed over the range, for in a few years the mean temperature for the month was abnormal, usually in a negative direction, as happened when the westerly air stream which usually warms our Islands broke down and was replaced by the cold continental climate. The outstanding example is that of February, 1947, when the mean temperature of 29.1° was -11.6° of the normal and -3.8° of the previous coldest February, that of 1929. Again, in January, 1940, the mean temperature was -9.9° of the normal, and -3.6° of the next lowest, in 1945. The months just mentioned are the only two in the records with a mean temperature of less than 32.0°.

At the other end of the scale, the mean temperature for the warmest January, in 1921, was $+7.0^{\circ}$ of the normal, while February's highest mean was recorded in 1926, with 46.7° or $+6.0^{\circ}$ of the normal.

The warmest month of any, July 1921, with a mean of 66.7°, was no more than $+5.0^{\circ}$ of the normal, and in only eight years has the variation from the normal for any month from May to October been greater than $\pm 4.0^{\circ}$. The least variation has occurred in May, when a range of $\pm 3.0^{\circ}$ of the mean covers all years but one, 1941, when the average of 48.4° was -4.6° of the normal, an outstanding figure.

Daily Temperature.

The highest daily maximum temperature ever recorded at Long Ashton is 93.0°, reached on two occasions, July 12th, 1924 and August 19th, 1932. In 1948 a maximum of 90° was recorded on consecutive days in July, the 28th and 29th.

Daily maxima of 80.0° have been recorded as early in the year as May, and as late as October. Temperatures over 80° have occurred five times in May, the highest being 84.0° on the 29th and 30th, 1944, and on the 5th and 6th of October, 1921 the maximum temperature was 80°. The daily maximum for March has been 70° or more in five years, the highest being 72.0° on the 29th and 30th in 1929.

The lowest minimum air temperature recorded was 6.0° on the 29th January, 1947. Two other instances of low temperature were on January 21st, 1940 and February 14th, 1929, with readings of 8.0° and 7.0° respectively.

Spring Frosts.

Air and ground frosts* severe enough to cause damage to fruit plants are likely to occur at any time during April, and in Table III it will be seen that though there are three years during the period 1931 to 1949 when there were no frosts during May, it is not until well into the second half of the month that the possibility of damaging frosts becomes remote.

TABLE III
GROUND FROSTS IN MAY DURING THE YEARS 1931-1949.

Year	No. of Ground Frosts	Latest Frost			Most Severe Frost		
		Day of month	Screen Min. (°F)	Grass Min. (°F)	Day of month	Screen Min. (°F)	Grass Min. (°F)
1931	2	21	34	28	21	34	28
1932	4	8	31	26	8	31	26
1933	0	—	—	—	—	—	—
1934	2	17	33	28	17	33	28
1935	5	19	32	28	17	28	21
1936	7	31	35	25	31	35	25
1937	0	—	—	—	—	—	—
1938	5	21	34	25	8	31	23
1939	1	7	37	29	7	37	29
1940	0	—	—	—	—	—	—
1941	12	18	35	28	16	28	18
1942	6	14	38	29	4	33	21
1943	4	11	32	23	11	32	23
1944	9	22	31	21	22	31	21
1945	3	4	32	27	1	25	17
1946	3	23	37	28	16	32	26
1947	1	4	40	29	4	40	29
1948	4	27	34	27	3	32	26
1949	4	10	32	25	10	32	25

The screen minimum of 25.0° recorded on the first of May, 1945, is the lowest air temperature for that month, and also equals the lowest air temperature for April, which has been registered on three occasions in that month during the period under review. The latest date on which air frost has been recorded is May 22nd, 1944, with a screen minimum of 31.0, while a grass minimum temperature of 25.0° occurred on May 31st, 1936, an unusually late date for a ground frost severe enough to cause damage to bush fruits. Slight ground frost has been registered on several occasions in June, the lowest reading being 28.0° on June 13th, 1939.

SUMMARY.

1. Records of the annual and monthly temperatures at Long Ashton during the years 1920-1949 are given.

2. During this period, the mean annual temperature has varied between 52.2° and 48.7°, the mean figure being 50.1°. An unusually warm year, 1921, was followed by a preponderance of cool years until 1933, which was the first of a series of seven years with temperatures above normal. The year 1949 was unusually warm.

3. The daily range of temperature is least during the winter months, but the greatest departures from the normal are to be found in January and February, particularly during cold winters.

4. The absolute daily maximum is 93.0°, recorded once in July and once in August. The absolute minimum is 6.0°, recorded in February.

5. Damage to plants may be caused by ground frost up to the end of May, but the possibility of serious loss is greatly reduced after the third week of the month.

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* A "ground frost" is regarded as having occurred when the thermometer on the grass registers 30.4° or below.

METEOROLOGICAL RECORDS 1949

METEOROLOGICAL RECORDS 1949																
Month	AIR TEMPERATURE (°F)						RAINFALL			SUNSHINE			SOIL TEMPERATURE at 09.00 hours			
	Mean		Dif. from average	Absolute Max.	Absolute Min.	Number of Ground Frosts	Total inches	Dif. from average	Most in a day	Total hours	Dif. from normal	Daily average	4"	8"	24"	
	Max. (A)	Min. (B)														
January	48.4	37.5	42.9	53	29	13	1.07	-2.48	0.30	57.3	+ 9.1	1.85	40.7	41.6	43.5	
February	50.1	35.3	42.7	55	23	18	1.46	-1.08	0.43	111.5	+ 44.3	3.98	39.8	40.6	42.4	
March	50.1	35.2	42.7	64	28	19	1.52	-0.82	0.46	140.5	+ 18.0	4.53	40.4	41.5	43.7	
April	57.9	43.6	50.4	74	30	4	2.25	0.0	0.66	166.4	+ 11.3	5.55	49.1	48.4	49.9	
May	61.3	43.5	52.5	71	32	4	3.48	+ 0.96	0.74	231.3	+ 29.7	7.46	56.9	54.0	54.2	
June	70.3	50.3	60.3	82	38	0	0.51	-1.63	0.26	282.7	+ 68.9	9.42	62.1	60.8	59.4	
July	74.6	54.6	64.6	88	47	0	1.42	-1.84	0.72	273.8	+ 79.7	8.83	67.1	66.0	64.3	
August	71.7	54.5	63.1	81	45	0	2.41	-1.00	1.56	216.9	+ 33.0	7.00	63.7	63.5	63.5	
September	69.1	53.8	61.5	81	45	0	2.52	-0.55	0.92	124.3	-19.0	4.14	60.5	61.0	62.2	
October	62.0	47.8	54.9	73	33	5	7.96	+ 4.08	1.19	114.6	+ 13.2	3.70	53.3	54.5	59.1	
November	50.6	38.9	44.8	58	28	13	3.58	+ 0.3	0.63	64.4	+ 8.1	2.15	43.1	44.4	50.0	
December	48.9	38.4	43.6	55	26	17	2.19	-1.60	0.37	54.1	+ 10.3	1.75	41.4	42.4	46.2	
Total or mean			52.0			93	30.37	-5.93		1837.8	+ 316.6	5.03	51.51	51.56	53.20	

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